The Journal of Laboratory and Clinical Medicine

VOL. 27 SEPTEMBER, 1942 NO. 12

CLINICAL AND EXPERIMENTAL

PREVENTION OF PORPHYRIN INCRUSTATIONS ON PANTOTHENIC ACID-DEFICIENT RATS BY HARDERIAN GLAND ABLATION*

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ONE of the most conspicuous symptoms observed in rats fed a diet deficient in pantothenic acid was the accumulation on the nose of an incrusted material that superficially resembled dried blood. This material appeared to emanate from the nostrils and was, therefore, observed most abundantly on the nose, whiskers, and fore paws, but it was sometimes spread to the fur of other parts of the body. The fur on the back of the neck region frequently appeared red or rusty. Because of the strong resemblance to dried blood and the apparent origin from the nose, this symptom had been described variously as "nosebleed,"[3] "blood-caked whiskers,"[18] "rusty spots on fur,"[9, 12] and "inflamed noses."[12]

Several months before the initiation of the work to be reported in this paper, it was observed in collaboration with others[10] that the red material on the rats exhibited a brilliant red fluorescence when illuminated with near ultraviolet light. Since blood is not fluorescent, an attempt was made to characterize this incrusted red fluorescent exudate chemically, and to discover its origin.

The red fluorescence made it appear probable that the incrusted material was a porphyrin (*see* Plate I, page 1608). Chemical and spectroscopic examination proved this to be the case. Other chemical tests indicated that very little, if any, of the fluorescent material was blood.[10] A survey of the literature re-

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This work was aided by a grant from the Anna Fuller Fund for Cancer Research.

The illustration for this article will be found in the section on Medical Illustration of this number of the JOURNAL, Plate I, page 1608.

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Received for publication, March 4, 1941.

vealed that the reddish incrusted exudate which appears on rats maintained on a filtrate factor deficient diet had been identified spectroscopically and described as protoporphyrin. The tests for blood were also negative.[2] Our own independent observations and more exhaustive investigation confirm this observation in some respects.[10]

At this stage of the investigation a short abstract appeared describing the harderian gland as a porphyrin-secreting gland.[6] The fact that the harderian gland was fluorescent and contained protoporphyrin had been known for some time.[4, 16] It thus appeared likely that the material was secreted by the harderian gland and passed to the nose via the nasolacrimal duct. In order to test this hypothesis, the harderian gland was completely removed from a number of rats to see if this operation would prevent the accumulation of the porphyrin-containing incrustations (*see* Plate I, page 1608). These operated animals and an equal number of controls were maintained on a diet deficient in pantothenic acid. This experiment demonstrated convincingly that harderian gland ablation prevents the porphyrin incrustations in albino rats maintained on a diet deficient in pantothenic acid. It is the purpose of this paper to present the details of this experiment.

METHODS AND MATERIALS

The 24 albino rats used in the experiment were weaned at the age of 22 to 24 days. They were from three litters* and, in so far as possible, the animals from each litter were uniformly distributed in four groups. There were 12 females and 12 males so that 3 of each sex were placed in each group of 6 rats (see Table I). The males and females were separated and placed three in a cage. The cages were made of pyrex glass and prevented contact of the animals or their excreta with metal. The feces, urine, and spilled food fell between the glass bars to pyrex glass plates and could not be retrieved by the rats. The feces were examined periodically to see whether they contained a red fluorescent material. The weights of the rats and other observations were recorded biweekly.

All rats were fed a basic diet of cerelose, 68 per cent; vitamin-free casein, 18 per cent; crisco, 8 per cent; salt mixture, 4 per cent; cod-liver oil, 2 per cent. This basic diet was supplemented for all rats by the addition of choline chloride (1 Gm. per kilogram) and the following vitamins: thiamine† (4 mg. per kilogram), riboflavin† (5 mg. per kilogram), pyridoxine† (4 mg. per kilogram), and nicotinic acid† (50 mg. per kilogram).[18] Twenty milligrams of pantothenic acid per kilogram were added to this for 12 animals maintained on a nondeficient diet. These mixtures were pounded compactly into the food cups to prevent excessive spilling. During the experiment special care was exercised to keep the water bottles filled at all times. This is an important requirement when investigating the porphyrin-containing incrustations.[5]

The harderian gland was removed from 11 rats (see Table I); five of these in one group were fed a diet deficient in pantothenic acid and 6 rats in another

*Obtained from the rat colony of the anatomy department through the courtesy of Dr. Charles W. Hooker.

†These vitamins were very kindly supplied by Dr. Robertson of Merck & Co.

group were fed the diet containing pantothenic acid. Rat 7 was not operated upon because at the time of operation it was thought that the animal would not survive the anesthesia. This unoperated animal did survive and was later regarded as a control for the two operated animals kept in the same cage.

The harderian gland in rodents is very large, occupying in the case of the rat about one-half of the total volume of the orbital cavity. This gland is a lacrimal gland. It is associated with the nictitating membrane and, therefore, is located on the medial side of the orbit. The location appears to be the chief characteristic used to distinguish it from the true lacrimal gland which is either extra-orbital or on the lateral side of the orbit. The harderian gland is horseshoe-shaped and almost completely surrounds the optic nerve and bulbus oculi.

TABLE I

INFLUENCE OF HARDERIAN GLAND ABLATION ON THE ACCUMULATION OF THE ENCRUSTED RED FLUORESCENT EXUDATE IN RATS

	ANIMAL		PORPHYRIN ENCRUSTATION	CONDITION OF FUR
	NO.	SEX		
Group 1A	1	F	Marked	Stained and matted
Not operated	2	F	Marked	Stained and matted
Pantothenic acid	3	F	Marked	Stained and matted
Deficient diet	4	M	Marked	Stained and matted
	5	M	Marked	Stained and matted
	6	M	Marked	Stained and matted
Group 1B	7*	F	Marked	Stained and matted
Operated	8	F	None	White and matted
Pantothenic acid	9	F	None	White and matted
Deficient diet	10	M	None	White and matted
	11	M	None	White and matted
	12	M	None	White and matted
Group 2A	13	F	Slight	Stained and fluffy
Not operated	14	F	Slight	Stained and fluffy
Pantothenic acid	15	F	Slight	Stained and fluffy
Nondeficient diet	16	M	None	Stained at times and fluffy
	17	M	None	Stained at times and fluffy
	18	M	None	Stained at times and fluffy
Group 2B	19	F	None	Very white and fluffy
Operated	20	F	None	Very white and fluffy
Pantothenic acid	21	F	None	Very white and fluffy
Nondeficient diet	22	M	None	Very white and fluffy
	23	M	None	Very white and fluffy
	24	M	None	Very white and fluffy

*Nonoperated.

The operations were performed with sodium amytal anesthesia (0.1 mg. per gram of rat weight). If necessary, this was supplemented with ether, but this practice has little to recommend it. The ether caused some animals to develop mucous plugs which gave rise to respiratory difficulties. In the first 3 animals two incisions were made between the eyes near the medial angles. The lateral edge was pulled laterally to expose the medial margin of the orbit. After the fascia attached to the medial margin of the orbit was cut, the gland was exposed. A suction pipette was applied to pull the gland out of the orbit and at the same time the blood vessels were cauterized. The blood vessels and other connections were then cut with scissors. During the operation the field was illuminated alternately with visible and near-ultraviolet light. This not only facilitated

locating the gland, but also insured the complete removal, because even a tiny bit of gland was so highly fluorescent that it was very conspicuous in near-ultraviolet light. A small portion of a harderian gland was found in the orbit on only one operated animal when the rats were killed and examined at the end of the experiment.

During the first few operations it was found that in order to remove completely the gland, the nictitating membrane and conjunctiva were frequently cut and damaged. In view of this, it appeared superfluous to make an incision in the skin. The last sixteen glands were removed by cutting the conjunctiva near the medial angle of the eye with an iridectome. The nictitating membrane and attached harderian gland were removed together. The other connections of the gland were cut with scissors, but no attempt was made to use a cautery. With this method the animals lost a drop or two of blood, but the eyes soon returned to normal. Since no incision was made in the skin, and no suturing was required, this was a much more rapid method. Examinations at the end of the experiment revealed only one eye damaged, and this was in one of the first animals operated upon. This injury was thought to have been caused by the cautery. Only one animal (No. 24) was blinded by the operation; the eyes of the other operated animals were normal. It was thought that all the animals would be blind from conjunctivitis caused by drying of the conjunctiva. The eyes of the operated animals were apparently kept sufficiently moist by the secretions from the extra-orbital lacrimal gland. This extra-orbital gland, located just in front of the ear, was found to exhibit a slight red fluorescence in only 6 of 50 rats examined specifically for this. No mention of the variable red fluorescence which was observed in this extra-orbital lacrimal gland was found in the literature. Even though there may have been some porphyrin in the secretion of some of these glands, it was never observed on the nose or fur of any of the animals with the harderian gland removed.

RESULTS

The experimental conditions for the different groups of animals and the observations regarding the porphyrin incrustations and condition of the fur were tabulated (see Table I). No fluorescent porphyrin-containing incrustations were observed at any time on animals in which the harderian gland had been removed. This was true regardless of the diet (group 1B, 2B). All nonoperated animals maintained on a diet deficient in panotothenic acid showed fluctuating, but marked, porphyrin incrustations (group 1A). These incrustations were red fluorescent and were most abundant on the nose, whiskers, and near the medial angle of the eye (*see* Plate I, page 1608). These symptoms required about four to five weeks to appear, and after this time varied from time to time, but in general became increasingly severe. Some of the nonoperated animals, maintained on a diet not deficient in pantothenic acid, exhibited slight and variable porphyrin incrustations. The slight porphyrin incrustations in this group were observed most frequently on the females. Operated animals maintained on a similar diet not deficient in pantothenic acid appeared unusually white and clean (group 2B) throughout the experiment. These very clear-cut

results indicate that the harderian gland is the source of the porphyrin-containing red fluorescent incrustations.

In addition to these observations several others were also recorded. An intense red fluorescence was observed in the feces of all nonoperated animals. In the feces of operated animals no red fluorescence was observed. The weight curves for the animals on the deficient diet were considerably below those on the nondeficient diet. Since these weights were found to agree with those of other investigators,[18] they have not been reproduced. The fur on all animals fed a diet deficient in pantothenic acid appeared matted. Depilation of body fur and bald head also occurred in some animals. The removal of the harderian gland did not influence these symptoms.

DISCUSSION

Previous investigations[4, 6, 16] had established the fact that the harderian gland contained and secreted porphyrin. The physiologic significance of the porphyrin in this fluid, which bathes the conjunctiva, has not been discovered. The anatomical pathway of this harderian gland secretion was followed by observing the red fluorescence. Under normal conditions the secretion was found to collect in the saccus lacrimalis and pass to the nasal cavity by way of the nasolacrimal duct. A fluorescent nasolacrimal duct was found in several cases. From the nose it may pass either to the alimentary tract directly via the nasopharynx or to the nose and whiskers through the external nares. In a normal rat on an adequate diet some of this red pigment was seen occasionally in visible light on the fur. When these normal rats were irradiated with near-ultraviolet light, a small amount of this red fluorescent material was frequently seen near the external nares. It was usually not very conspicuous, however, as healthy rats keep it cleaned off by washing and licking.

In some rats porphyrin incrustations were induced by water deprivation. When these rats were given water, they drank it and soon cleaned the incrusted material off by licking and washing. The rats were killed two to four hours later. A red fluorescent band was observed in the intestine. It was assumed that this band was caused by the unusually heavy dose of concentrated porphyrin cleaned off the nose and fur by washing. This may account for the presence of large amounts of porphyrin which had been observed in the excreta of rats.[13] The red fluorescence of the feces was not observed in the case of the operated animals. This is no proof, but it suggests that the harderian gland may elaborate the porphyrin which it secretes.

In the case of nonoperated rats, maintained on diets deficient in pantothenic acid, this porphyrin-containing secretion accumulated and became incrusted on the nose, whiskers, and face. The infrequent and feeble attempts to remove this by washing and licking were unsuccessful and usually the material was merely smeared around to other areas, notably the fore paws and back of the neck. This smearing and unsuccessful cleaning, while partly caused by the lethargy of the rat, was also thought to be related to a change in the physical nature and the amount of the saliva. It appeared to function like a liquid glue that did not dissolve or remove the material, but acted as an adhesive base for the smeared porphyrin-containing excretion.

Much evidence was found to support this concept of the mechanism of the accumulation of porphyrin incrustations. Water deprivation and limitation induced porphyrin incrustations in rats.[5] This had been observed previously, but the red exudate was not only mistaken for blood, but the tests for blood were positive.[15] Pantothenic acid deficiency may influence water metabolism indirectly through its effect on the adrenals. A deficiency of this vitamin in the diet caused adrenal cortical damage and necrosis.[1, 3, 11, 14] Experimental adrenal cortical insufficiency resulted in the loss of water from the plasma and general dehydration as indicated by hemoconcentration in cats and decreased plasma volume in dogs.[8] The rats fed a deficient diet sneezed more frequently and drank less water (approximately one-half as much) than the animals on a nondeficient diet. It was thought that this sneezing might be an indication of a relatively dry nasal mucous membrane.

The porphyrin-containing exudate which was seen to come from the nose was doubtless a mixture of the harderian gland and nasal secretions. Any changes in physical properties of these secretions that would result from a general dehydration would also have to be regarded as an important factor in the control of the adhesive properties of this exudate. From these bits of evidence gathered from various sources, it may be seen that pantothenic acid deficiency caused adrenal necrosis[1, 3, 11, 14] which, according to the work of Lewis,[8] caused a loss of water from the plasma and general dehydration. Water deprivation and consequent dehydration caused a marked decrease in salivary flow in man and dogs,[7] and both pantothenic acid deficiency and water deprivation induced the porphyrin incrustation symptom in rats.[5] These facts indicate that pantothenic acid deficiency regulates water metabolism; it suggests the mechanism by which it does this, and influences the accumulation of porphyrin incrustations.

SUMMARY

Ablation of the harderian glands prevents the accumulation and incrustation of a red fluorescent porphyrin-containing exudate in rats fed a diet deficient in pantothenic acid. This indicates that the harderian gland is the immediate glandular source of the porphyrin in the incrusted material observed on the nose, whiskers, and fur of rats fed a diet deficient in pantothenic acid.

REFERENCES

1. Ashburn, L. L.: The Effect of Administration of Pantothenic Acid on the Histopathology of the Filtrate Factor Deficiency State in Rats, Pub. Health Rep. **55:** 1337, 1940.
2. Chick, H., Macrea, T. F., and Worden, A. N.: Relation of Skin Lesions in Rats to Deficiency in Diet of Different B_2 Vitamins, Biochem. J. **34:** 580, 1940.
3. Daft, F. S., Sebrell, W. H., Babcock, S. H., Jr., and Jukes, T. H.: Effect of Synthetic Pantothenic Acid on Adrenal Hemorrhage, Atrophy, and Necrosis in Rats, Pub. Health Rep. **55:** 1333, 1940.
4. Derrien, E., and Turchini, J.: Sur l'accumulation d'une porphyrine dans la gland des Harder des Rongeurs du genre Mus et sur son mode d'excretion, Compt. rend. Soc. de biol. **91:** 637, 1924.
5. Figge, F. H. J., and Atkinson, W. B.: Relation of Water Metabolism to Porphyrin Incrustations in Pantothenic Acid-Deficient Rats, Proc. Soc. Exper. Biol. & Med. **48:** 112, 1941.
6. Grafflin, A. L.: Porphyrin-Secreting Harderian Gland of the Albino Rat, Anat. Rec. **79:** 25 (abst.), 1941.
7. Gregersen, M. I., and Bullock, L. T.: Observations on Thirst in Man in Relation to Changes in Salivary Flow and Plasma Volume, Am. J. Physiol. **105:** 39 (abst.), 1933.

8. Lewis, L. A.: The Blood Picture of Adrenalectomized Animals Treated With Different Adrenal Fractions, Endocrinology **28**: 821, 1941.
9. Lunde, G., and Kringstad, H.: Über Veränderungen des Pelzes von Ratten durch Mangel an gewissen Faktoren des Vitamin B-Komplexes, Hoppe Seyler's Ztschr. f. physiol. Chem. **257**: 201, 1939.
10. McElroy, L. W., Salomon, K., Figge, F. H. J., and Cowgill, G. R.: On the Porphyrin Nature of the Fluorescent "Blood Caked" Whiskers of Pantothenic Acid Deficient Rats, Science **94**: 467, 1941.
11. Morgan, A. F., and Simms, H. D.: Adrenal Atrophy and Senescence Produced by a Vitamin Deficiency, Science **89**: 565, 1939.
12. Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B.: Additional Nutritional Factors Required by the Rat, J. Biol. Chem. **127**: 23, 1939.
13. Rimington, C., and Hemmings, A. W.: Porphyrinuria Following Sulphanilamide: Sulfanilamide Dermatitis, Lancet **1**: 770, 1938.
14. Salmon, W. D., and Engel, R. W.: Pantothenic Acid and Hemorrhagic Adrenal Necrosis in Rats, Proc. Soc. Exper. Biol. & Med. **45**: 621, 1940.
15. Smith, S. G., and Sprunt, D. H.: Pathological Skin Changes in the Tail of the Albino Rat on a Diet Deficient in Vitamin G, J. Nutrition **10**: 481, 1935.
16. Thomas, M. J.: Contributions à l'étude des porphyrines en biologie et en pathologie. I. Genèse du noyau tétrapyrrolique, Bull. Soc. Chim. biol. **20**: 471, 1938.
17. Unna, K.: Effect of Pantothenic Acid on Growth and Reproduction of Rats on Synthetic Diets, Am. J. M. Sc. **200**: 848, 1940.
18. Unna, K.: Pantothenic Acid Requirement of the Rat, J. Nutrition **20**: 565, 1940.

DUODENAL BULB ("ULCER-BEARING AREA") ACIDITY IN FASTING NORMAL PEOPLE*

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IN THE present-day concept of the problem of chronic peptic ulcer the acid gastric juice occupies a position of acknowledged prominence. Since the gastric acid is important only because of its effects, direct and indirect, either on the ulcer proper or on some area susceptible to ulcer, one wonders why attention has been directed so exclusively to acidity in the stomach when most clinical ulcers occur in the duodenum.[4] Simultaneous determinations of gastric and duodenal acidity would serve better to evaluate the accepted importance of the acid gastric juice in duodenal ulcer. However, our knowledge of the changes in acidity which occur in the "ulcer-bearing" duodenal bulb is very limited.[16, 26] We particularly need more extensive information concerning the situation which normally exists in the duodenal bulb before we can hope to interpret the findings in ulcer patients.

In a previous study[8] we found, in agreement with Morton,[26] that the contents of the duodenal bulb in normal people following an Ewald meal were endowed with a considerable capacity to neutralize, buffer, and dilute the gastric chyme. Several investigators,[13-15, 19, 25-27] using men as subjects, had commented on the apparent lack of close relationship between gastric and duo-

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Aided by a grant from John Wyeth & Brothers, Inc.

A portion of a thesis submitted by Dr. Berk to the Faculty of the Graduate School of Medicine of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Medical Science, D.Sc. (Med.), for graduate work in internal medicine.

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denal acidity and we were able to demonstrate a similar lack of sharp parallelism between the acidity in the stomach and that present at the same moment in the first part of the duodenum of normal persons after an Ewald meal.

The present study was undertaken to determine whether a neutralizing efficiency of equal magnitude and relationships of like nature obtained in the duodenal bulb during fasting in normal people studied under the same experimental conditions.

MATERIAL

The subjects utilized for the purposes of this study were drawn largely from the Out-Patient Gastro-Intestinal Clinic of the Jefferson Hospital. Although a number of them presented symptoms referable to the digestive tract, none gave evidence of fever or of any acute, chronic, infectious, metabolic or cachectic disease, and x-ray examination revealed no abnormality of the stomach and duodenum. Achlorhydries were not excluded, since a lack of free acid in the stomach is expected in a certain percentage of "normal" persons.[2, 3, 20, 23, 24, 29, 30, 35]

We could not hope to standardize all the variables which are known to affect gastric secretory behavior even in "normal" people[8] and, inasmuch as our primary concern was with the changes in acidity in the duodenal bulb which might be associated with changes in gastric acidity, we felt that variations in gastric acidity were actually desirable for our purpose.

Twenty-two subjects were studied, of which 14 were males and 8 were females. The age of the entire group ranged from 22 to 51 years, with an average age of 35.5 years. The males ranged from 22 to 51 years of age, with an average age of 32.5 years; the females from 29 to 49 years of age, with an average age of 40.9 years.

METHOD

Each subject was examined in the morning before breakfast, following a period of alimentary rest of at least twelve hours. A specially constructed double lumen tube was employed, and a method was utilized which permitted more or less fluoroscopic control as well as radiographic proof of the position of the tube.[5] Most of the subjects had swallowed tubes before for other purposes so that the element of fright was minimal. If much difficulty was encountered in swallowing the tube, if nausea or vomiting occurred, or if the subject complained of any other distress of any moment, the experiment was discontinued, and the results were disregarded. After ascertaining the location of the tube in the stomach with the aid of the fluoroscope, we were able, in some instances, to manipulate the tube into position in the duodenum. When we failed in this attempt, the subject was placed on his right side, the room was darkened, and the person was exhorted to relax and slowly swallow about 6 inches of the tube over a period of half an hour; not uncommonly the subject was asleep when we returned to the room at the end of that period.

Once the tube was determined by fluoroscopic examination to be in the desired position, the person was placed at rest on his right side, and the aspiration apparatus was assembled. Specimens were collected simultaneously from

the pars pylorica and the duodenal bulb at ten-minute intervals for one-half hour. At each of the four-interval aspirations all the material which could be obtained was collected.

The pH of each specimen was determined at once by means of a Leeds-Northrup pH indicator, which uses a glass electrode. After filtration the free and total acidity of each specimen was estimated using Töpfer's reagent and phenolphthalein as the respective color indicators.[6] In addition, on each duodenal specimen, we determined what was called the excess neutralizing ability.[4, 6] This consisted of the amount of N/10 hydrochloric acid necessary to lower the pH to the point at which Töpfer's reagent evidenced a positive reaction for free acid.[6]

Out of 29 experiments 25 were accepted as being satisfactory with respect to technique. These involved 558 separate determinations made up of 156 pH readings, 163 estimations of free acid, 163 estimations of total acidity, and 76 determinations of excess neutralizing ability of the duodenal contents.

RESULTS

Acidity in pH Units (Fig. 1).—Stomach. The average gastric acidity in terms of pH in fasting normal subjects (pH 3.51) was less than that observed after an Ewald meal (pH 2.78),[8] even though the range of the individual pH values in both the fasting and digestive states was about equal. It is interesting to note that even the average gastric acidity expressed in pH units of those subjects who had some free acid in their fasting stomach contents (pH 2.37) was less than that in fasting normal dogs (pH 1.90).[7]

Duodenum. The efficient neutralizing ability of the contents of the duodenal bulb during fasting was evidenced by the constant maintenance of a difference in pH in the samples collected at the same moment from areas just above and just below the pylorus. Throughout the period of observation in the fasting state this difference averaged 2.09 pH units. Further evidence of the effectiveness of duodenal neutralization in the fasting state was found in the relative infrequency of occurrence of free acid in the duodenum. Under fasting conditions only 26 per cent of all the samples had a pH less than 3.5, the critical value we had adopted for free acid,[6] compared with 31.7 per cent during the observation period following an Ewald meal.

A peculiar feature, which was seen in the entire group as well as in those members of this group who were able to secrete free acid, was a progressive elevation in the average duodenal pH during the period of observation. As a result, the character of the line representing the plotted values for average duodenal pH was distinctly different in pattern from the corresponding one for average gastric pH.

The over-all average duodenal pH was 5.60, which was about equal to that we found in fasting normal dogs (5.56),[7] despite a much greater acidity in terms of pH in the stomach of dogs as compared with men. The much more efficient duodenal bulb neutralization in fasting dogs was also seen in the fact that only 17.2 per cent of the duodenal samples in these animals showed free acid (a pH less than the critical value of 3.5).

Free Acid (Fig. 2).—Stomach. The average gastric free acid in fasting normal subjects was 15 clinical units. This was less than that after an Ewald

meal[8] (21 clinical units), and the maximal values attained were not as great. The average gastric free acid was also much less than that in fasting normal dogs.[7] There was a contrast between the configuration of the line representing the plotted values of average gastric free acid and that representing average duodenal pH (Fig. 1).

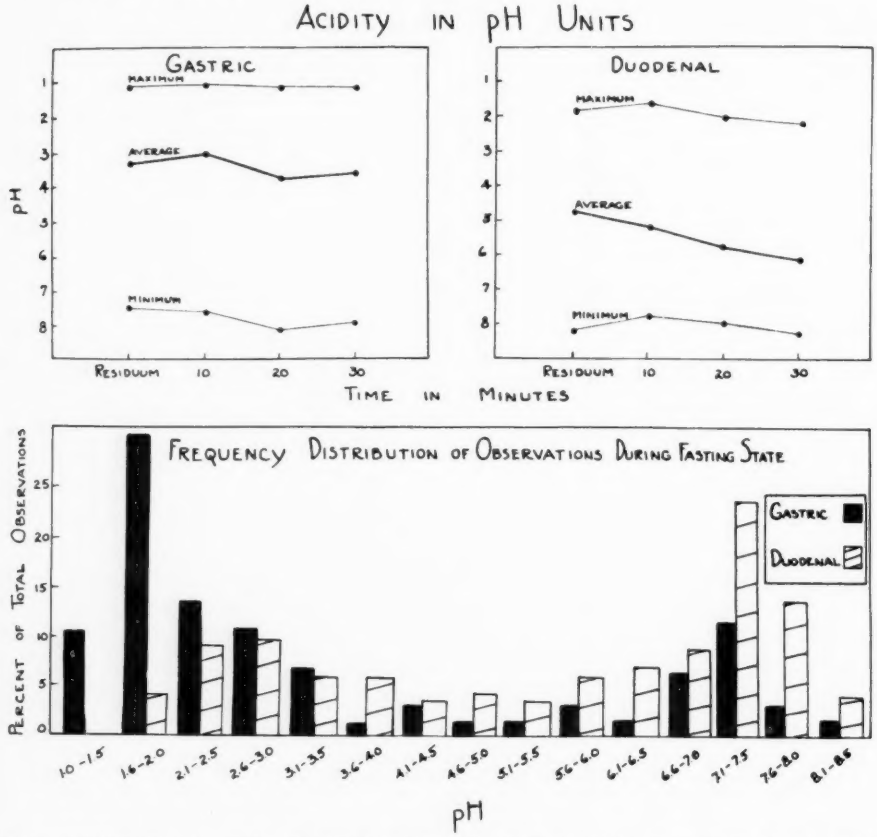


Fig. 1.—Acidity in pH units of samples collected simultaneously from just above and just below the pylorus in fasting normal people.

Duodenum. In the vast majority of instances the contents of the duodenal bulb under fasting conditions showed an absence of free acid; only 10 per cent of all the specimens yielded a colorimetric reaction with Töpfer's reagent, such as was considered positive for free acid. A greater percentage of these samples (26 per cent), it will be recalled, were thought to contain free acid as judged by their pH values (Fig. 1). The apparent discrepancy between these percentages may be attributed to our method of filtration and dilution preparatory to colorimetric titration which results in a number of false negative readings.[6] Our end point with Töpfer's reagent was about pH 3.5,[6] so that a more accurate figure is the one based upon the assumption that all specimens whose pH in the unfiltered, undiluted state is 3.5 or less contain free acid.

The percentage of duodenal samples positive for free acid was less than in normal people after an Ewald meal in whom free acid was found col-

orimetrically in 15.2 per cent, and electrometrically in 31.7 per cent of the samples.[8] An added contrast indicating the very effective neutralizing power of the contents of the duodenal bulb during fasting was the smaller percentage of subjects who had free acid in the contents of their bulb at some time during the period of observation. The comparative values are: fasting, 22.7 per cent of the subjects (colorimetrically), 40.9 per cent (electrometrically); Ewald

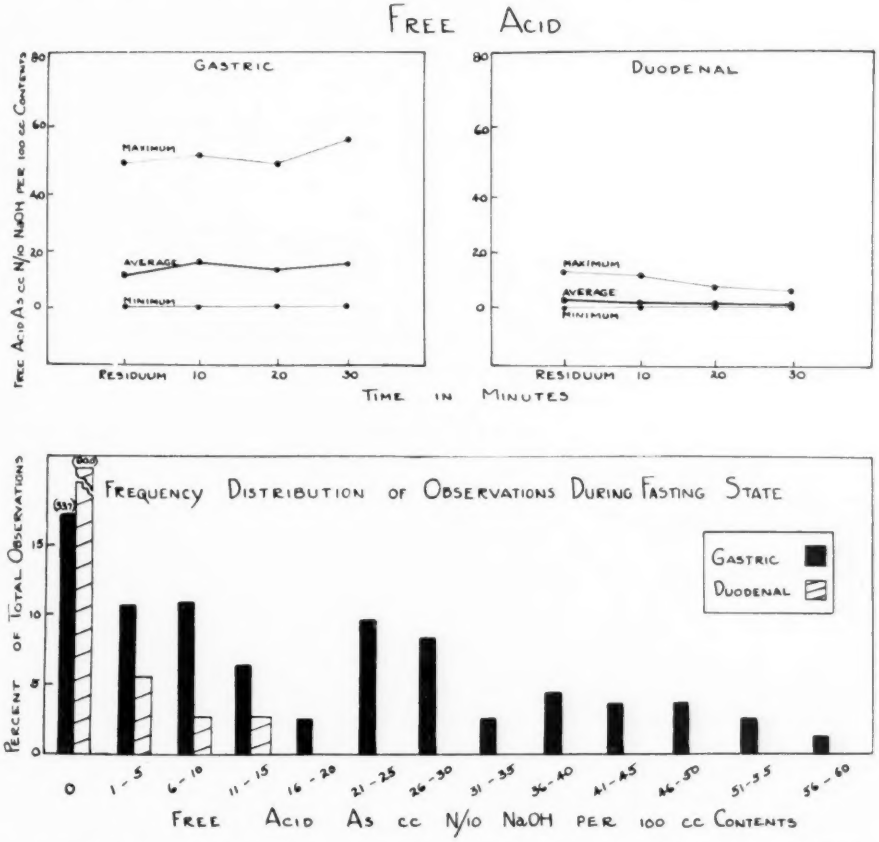


Fig. 2.—Free acid as determined on samples collected simultaneously from just above and just below the pylorus in fasting normal people.

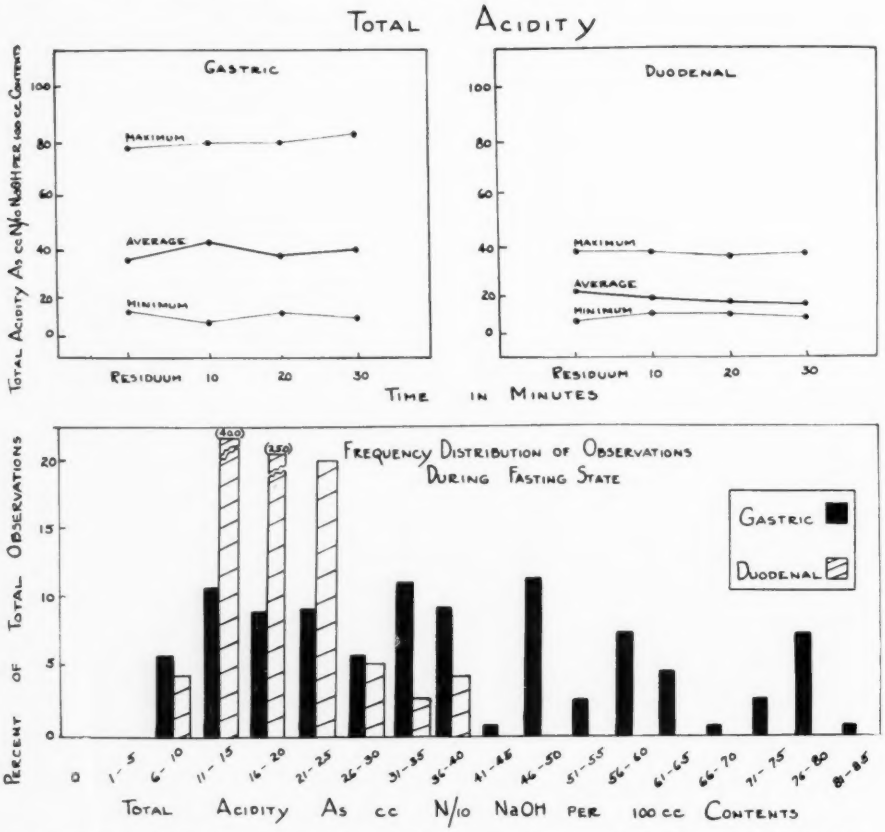
meal, 66.6 per cent of the subjects (colorimetrically), 75 per cent (electrometrically). On the other hand, duodenal neutralization in fasting normal persons was less effective than in normal fasting dogs[7] despite a greater gastric acidity in the latter animals; colorimetrically, only 6.3 per cent, and electrometrically, only 17.2 per cent of the duodenal samples were positive for free acid in fasting dogs.

What little free acid was contained in the duodenal bulb contents during fasting showed the same tendency toward a diminution in average values with each successive aspiration, as had been noted in the case of duodenal pH (Fig. 1).

Total Acidity (Fig. 3).—Stomach. Average gastric total acidity, as with average gastric free acid, was distinctly less than that in normal people following an Ewald meal.[8] It was also less than in normal fasting dogs.[7] A differ-

ence in configuration of the graphic curves of average gastric total acidity and average duodenal pH was evident (Fig. 1).

Duodenum. The average value for duodenal total acidity was 18 clinical units. This and the narrower range of distribution of the individual values under fasting conditions indicate a neutralizing capacity at least equal to that observed in normal people after an Ewald meal.[8] The average value and the range of values, however, were not much less than those in fasting normal dogs in spite of the greater gastric acidity in the latter.[7]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GASTRIC
DUODENAL
MAXIMUM
AVERAGE
MINIMUM
TOTAL ACIDITY AS cc N/10 NaOH PER 100 cc CONTENTS
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GASTRIC
DUODENAL
PERCENT OF TOTAL OBSERVATIONS
0 1-5 6-10 11-15 16-20 21-25 26-30 31-35 36-40 41-45 46-50 51-55 56-60 61-65 66-70 71-75 76-80 81-85
TOTAL ACIDITY AS cc N/10 NaOH PER 100 cc CONTENTS

Fig. 3.—Total acidity of samples collected simultaneously from just above and just below the pylorus in fasting normal people.

Here again the contents of the duodenal bulb during fasting displayed the rather characteristic trend toward progressive decrease in acidity values. The line representing the plotted values for average duodenal total acidity assumed a form which was quite dissimilar in pattern from the corresponding curve for average gastric total acidity.

Neutralizing Ability of the Duodenal Contents (Fig. 4).—We previously defined excess neutralizing ability as a measure of the reserve capacity which the contents of the first part of the duodenum possess to neutralize, buffer, and dilate the secretions received from the stomach above that necessary to offset the free acid content,[4, 6]

The contents of the duodenal bulb in the fasting state slightly surpassed those in the digestive state, as measured by this index of neutralizing capacity. The over-all average was 17 clinical units, which was a little higher than after an Ewald meal (14 clinical units).[8] Only 14.5 per cent of all the samples had no excess neutralizing ability, as compared with 20 per cent of those after an Ewald meal. As measured by the same index, however, the neutralizing capacity was inferior to that in fasting normal dogs;[7] in the latter the over-all average value was greater (28 clinical units), the range of distribution of the individual values was wider, and the percentage of specimens negative, for excess neutralizing ability was less (8 per cent).

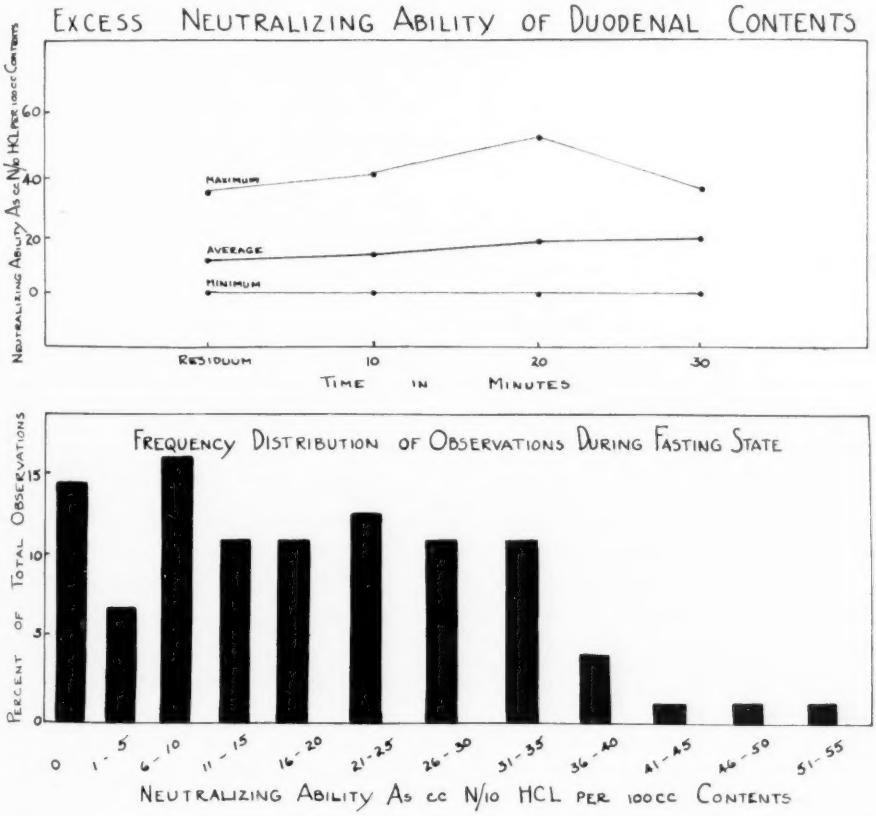

Fig. 4.—Excess neutralizing ability of the duodenal contents in fasting normal people.

The fairly typical trend toward a progressive decrease in acidity in the duodenal bulb with each succeeding aspiration was seen here in the slight but definite increase in values for average excess neutralizing ability.

DISCUSSION

The figures we obtained in the course of this study are not submitted with the thought that they establish an ultimate standard for the acidity in the duodenal bulb in all fasting normal people. Despite our efforts to control the origin of the specimens, undoubtedly some of them must have been drawn from portions of the stomach and duodenum other than those which were designated.

The existence of a true fasting gastric secretion in men is still a moot question.[1, 9, 11, 28, 31-33] Even though we made an attempt to avoid all extraneous influences and to secure a true resting state, it is unlikely that all normal and abnormal stimuli sufficient to influence gastric secretion were thoroughly excluded.[1] The values for fasting gastric acidity which we obtained, while in agreement with those observed by others with respect to both pH[10, 16, 21, 22] and titrable acidity,[2, 3, 17, 18, 32, 33, 34] are not advanced as necessarily characteristic of the normal fasting stomach. The findings, however, are useful for comparison with like values simultaneously obtained in the duodenal bulb or with corresponding values obtained under similar experimental conditions during the course of digestion.

Under our experimental conditions the reaction in the first part of the duodenum in man is acid during the fasting state, just as it is during the digestive state. In a thirty-minute observation period the contents of the duodenal bulb displayed a more or less characteristic tendency toward a progressive decrease in average acidity values, and a concomitant progressive increase in neutralizing ability. This feature was all the more interesting because it occurred independently of any similar trend in gastric acidity.

The contents of the "ulcer-bearing" duodenal bulb in fasting normal people are able to neutralize, buffer, and dilute the gastric secretions as well, and possibly even better, than during the course of digestion of an Ewald meal. A difference in reaction is constantly maintained between the adjacent pars pylorica and duodenal bulb which about equals that during digestion. While neutralization is generally effective (measured by the ability to maintain a pH of 3.5 or above), free acid is by no means constantly or consistently absent. Therefore, as in the digestive state so in the fasting state as well, the occasional presence of free acid in the contents of the duodenal bulb cannot be considered as greatly significant from the diagnostic standpoint.[12, 14, 15, 19, 26]

The frequently noted lack of sharp parallelism between the several indexes of gastric acidity on the one hand, and the duodenal pH as well as the corresponding indexes of duodenal acidity on the other, permits the conclusion that there is no constant relationship between the gastric acidity and that present at the same time in the duodenal bulb.

The fact that neutralization of gastric acid in the duodenal bulb is apparently more efficient in dogs than in man may be related to the fact that dogs rarely, if ever, display a spontaneous duodenal ulcer, whereas, man is very frequently afflicted with this disturbance.

SUMMARY AND CONCLUSIONS

1. The "ulcer-bearing" first portion of the duodenum in fasting normal people is an acid area with an average pH of 5.60.

2. The duodenal bulb in fasting normal people is able to neutralize, buffer, and dilute the gastric secretions with an efficiency which equals and possibly exceeds that during digestion of an Ewald meal.

3. Free acid is generally, but not consistently, absent in the contents of the duodenal bulb in fasting normal persons; its presence cannot be taken as a necessarily abnormal finding.

4. There is no constant relationship maintained between the acidity simultaneously determined in the stomach and in the duodenal bulb.

5. Fasting normal dogs display a greater neutralizing power in the duodenal bulb than do fasting normal men.

We wish to express our thanks to Dr. B. B. Vincent Lyon for his kind permission to use the facilities of the Gastro-Intestinal Clinic, and to Drs. Melvin Dillman and Karl Kornblum for their cooperation in performing the x-ray studies on each subject.

REFERENCES

1. Babkin, B. P.: Does the Stomach Secrete Gastric Juice Continuously? Libman Anniversary **1**: 113, 1932.
2. Baird, M. Mc., Campbell, J. M. H., and Hern, J. R. B.: Gastric Secretion, Physique and Physical Fitness, Guy's Hosp. Rep. **74**: 339, 1924.
3. Bennett, T. I., and Ryle, J. A.: Studies in Gastric Secretion. V. A Study of the Normal Gastric Function Based on the Investigation of 100 Healthy Men by Means of the Fractional Method of Gastric Analysis, Guy's Hosp. Rep. **71**: 286, 1921.
4. Berk, J. E., Thomas, J. E., and Rehfuss, M. E.: The Reaction and Neutralizing Ability of the Contents of the Pyloric Antrum and First Part of the Duodenum in Normal Dogs Following an Ewald Meal, Am. J. Physiol. **136**: 157, 1942.
5. Berk, J. E., Rehfuss, M. E., and Thomas, J. E.: A Method for the Simultaneous Aspiration of the Contents of the Stomach and First Part of the Duodenum, J. A. M. A. **119**: 259, 1942.
6. Berk, J. E., Thomas, J. E., and Rehfuss, M. E.: Limitations in the Use of Color Indicators in Gastric Analysis, Am. J. Digest. Dis. **9**: 106, 1942.
7. Berk, J. E., Thomas, J. E., and Rehfuss, M. E.: The Reaction and Neutralizing Ability of the Contents of the First Part of the Duodenum in Normal Dogs Under Fasting Conditions, Am. J. Physiol. **136**: 369, 1942.
8. Berk, J. E., Rehfuss, M. E., and Thomas, J. E.: The Acidity of the "Ulcer-Bearing Area" of the Duodenum in Normal Persons, Am. J. Digest. Dis. In press.
9. Bloomfield, A. L., Chen, C. K., and French, L. R.: Basal Gastric Secretion as a Clinical Test of Gastric Function With Special Reference to Peptic Ulcer, J. Clin. Investigation **19**: 863, 1940.
10. Breuhaus, H. C., and Eyerly, J. B.: Antacids: Their Effect by Titration and Within the Human Stomach, Ann. Int. Med. **14**: 2285, 1941.
11. Carlson, A. J.: The Secretion of Gastric Juice in Health and Disease, Physiol. Rev. **3**: 1, 1923.
12. Einhorn, M.: Experiences With the Duodenal Contents, J. A. M. A. **56**: 6, 1910.
13. Einhorn, M.: The Fractional Examination of the Duodenal Contents, Am. J. M. Sc. **156**: 817, 1918.
14. Einhorn, M.: Fractional Examination of the Duodenal Contents in Peptic Ulcer, J. A. M. A. **77**: 1471, 1921.
15. Einhorn, M.: The Duodenal Tube and Its Possibilities, Philadelphia, 1926, F. A. Davis Co.
16. Eyerly, J. B.: Comparative pH Values Within the Stomach, Pylorus and Duodenum in Antacid Therapy, Am. J. Digest. Dis. **7**: 431, 1940.
17. Fowler, C. C., Rehfuss, M. E., and Hawk, P. B.: Gastro-intestinal Studies. X. An Investigation of the Gastric Residuum in Over 100 Normal Cases, J. A. M. A. **65**: 1021, 1915.
18. Fowler, C. C., and Zentmire, Z.: Studies of the Gastric Residuum. I. A Study of 80 Samples of Gastric Residuums Obtained From Apparently Normal Women, J. A. M. A. **68**: 167, 1917.
19. Friedenwald, J., and Sindler, J.: Fractional Analysis of the Duodenal Contents in Normal Individuals, J. A. M. A. **77**: 1469, 1921.
20. Gershon-Cohen, J., Shay, H., and Fels, S. S.: Experimental Studies on Gastric Physiology in Man. VI. The Relation of Size, Shape and Position of the Stomach to Its Acid Secretion, Am. J. Roentgenol. **40**: 695, 1938.
21. Helmer, O. M., Fouts, P. J., and Zerfas, L. G.: Gastro-Intestinal Studies. I. Gastric Juice in Pernicious Anemia, J. Clin. Investigation **11**: 1129, 1932.
22. Helmer, O. M., Fouts, P. J., and Zerfas, L. G.: Gastro-Intestinal Studies. IV. The Relation of pH to the Pepsin and Rennin Content of the Gastric Juice, Am. J. Digest. Dis. **1**: 120, 1934.
23. Keefer, C. S., and Bloomfield, A. L.: The Significance of Gastric Anacidity, Bull. Johns Hopkins Hosp. **39**: 304, 1926.
24. Lerman, J., Pierce, F. D., and Brogan, A. J.: Gastric Acidity in Normal Individuals, J. Clin. Investigation **11**: 155, 1932.
25. McClure, C. W., and Wetmore, A. S.: Studies in Pancreatic Function, Enzyme Concentration of Duodenal Contents After the Ingestion of Pure Foodstuffs and Food Mixtures by Normal Men, Boston M. & S. J. **187**: 882, 1922.

26. Morton, C. B.: (a) Observations on Peptic Ulcer. VI. Preliminary Report of Clinical Experiments With Gastro-Duodenal Analysis, Am. J. M. Sc. **177**: 65, 1929; (b) Observations on Peptic Ulcer, South. Surgeon **3**: 316, 1934.
27. Okoda, S., and Arai, M.: The Hydrogen Ion Concentration of the Intestinal Contents, J. Biol. Chem. **51**: 135, 1922.
28. Pavlov, I. P.: The Work of the Digestive Glands, London, 1910, J. B. Lippincott Co.
29. Polland, W. S.: Histamine Meals. An Analysis of 988 Consecutive Tests, Arch. Int. Med. **51**: 903, 1933.
30. Polland, W. S., and Bloomfield, A. L.: Unexplained Gastric Anacidity, Arch. Int. Med. **48**: 412, 1931.
31. Polland, W. X., and Bloomfield, A. L.: Basal Gastric Secretion in Man, Bull. Johns Hopkins Hosp. **49**: 302, 1931.
32. Rehfuss, M. E., Bergeim, O., and Hawk, P. B.: Gastro-Intestinal Studies. I. The Question of the Residuum Found in the Empty Stomach, J. A. M. A. **63**: 11, 1914.
33. Rehfuss, M. E.: The Diagnosis and Treatment of Diseases of the Stomach, Philadelphia, 1927, W. B. Saunders Co.
34. Talbot, E. S.: The Examination of Normal Gastric Secretion by the Fractional Method, J. A. M. A. **66**: 1849, 1916.
35. Vanzant, F. R., Alvarez, W. C., Eusterman, G. B., Dunn, H. L., and Berkson, J.: The Normal Range of Gastric Acidity From Youth to Old Age, Arch. Int. Med. **49**: 345, 1932.

ACUTE POSTOPERATIVE LUNG ABSCESS

Report of a Case With Unusual Bacteriologic Findings

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INFECTION is one of the prerequisites of the breakdown of lung tissue. The infecting organism in some cases is very specific, as in the case of a tubercular cavity or an amebic abscess of the lung. These are usually not considered under the heading of lung abscesses; however, the primary acute putrid or acute nonputrid abscesses of the lung are those that are grouped under this name.

There are observers of great experience in this field, among them Neuhof,[1] who readily ascribe all nonputrid abscesses to aerobic bacteria, and all putrid abscesses to anaerobic ones. Nevertheless, papers by Bucher,[2] Cohen,[3] and Varney,[4] with extensive bacteriologic studies, cast some doubt on the truth of this simple classification. Cohen[3] reported the results of an exhaustive bacteriologic study of 16 cases; he emphasized that it is highly debatable whether anaerobes are the determinant feature of putrid lung abscess. He showed that gamma streptococcus and diphtheroids were present in each of the 16 examined cases, and *Bacillus melaninogenicum* was observed in 15 of these. A great variety of other bacteria occurred in varying numbers. The findings of Bucher,[2] in a larger but seemingly not so meticulously studied group, indicated a total incidence of 451 bacteria in 118 cases of lung abscess. Three streptococcus groups described as hemolytic, nonhemolytic, and *viridans*, occurred 93 times. The next in frequency was the *Micrococcus catarrhalis*, occurring 61 times; diphtheroids, on the other hand, were found only 29 times, and the *Bacillus melaninogenicum* is not mentioned in his report. Varney[4] reported 27 cases in which certain rigid requirements were adhered to in order to obtain

all, but no other organisms than those actually present in the abscess. He stressed the point that anaerobes are found mostly in granules occurring in pus of abscesses. In two instances out of a total of 27 all the growth was anaerobic; in the other 25 there were both aerobes and anaerobes present.

These reports bring out the fact that usually more than one bacterial species is found in a lung abscess and also, that in many cases aerobes and anaerobes occur, irrespective of whether the abscess is putrid or not. A lung abscess harboring only one species of bacterium seems to be rather rare. It is of interest, therefore, to report a case of acute, nonputrid postoperative lung abscess in which two cultures were obtained from the pus aspirated from the lung. The pus was obtained before perforation into the bronchus, or operative drainage would have taken place. Both cultures showed that the only bacterium present was a micro-aerophilic, nonhemolytic, gram-positive streptococcus. The case also presented other interesting features.

G. E., female, married, 24 years of age, came under observation in November, 1940. Two years prior to this date she underwent an appendectomy for right lower quadrant pain; following this operation, the pain persisted and became her main complaint.

She had one child and had had no miscarriages. Her menstrual periods came every 35 days and were of seven days' duration; usually the pain in the right lower quadrant became more intense during the period. She had a "streptococcic" sore throat in 1939, and as later verified from the physician in charge, this diagnosis was made on clinical bases alone. Her weight was 102 pounds.

On examination the essential findings were as follows: wide diastasis of the recti muscles; small mass on the right side of the pelvis, thought to be due to salpingo-oophoritis, and cervical erosion. Treatment consisted of cauterization of the cervix, followed by biweekly diathermy and aolan injections. This regime was maintained until Jan. 17, 1941. There was no appreciable relief. The patient had an upper respiratory infection from which she apparently recovered clinically.

She was admitted to the Newark Beth Israel Hospital on Feb. 9, 1941, and was operated on, under general anesthesia, the following day. The operation consisted of right salpingo-oophorectomy, resection of the presacral nerve, and reconstruction of the abdominal wall. She stood the procedure well.

On the fourth postoperative day a nonproductive, spastic, and persistent cough developed, the temperature rose to 102° F., but the physical findings were essentially negative. An expectorant was prescribed. The cough continued, became loose and annoyingly persistent. The maximum temperature on the eighth and ninth postoperative days did not exceed 100° F., and the patient was more or less comfortable. On the eleventh postoperative day, however, the cough reappeared, the temperature rose again, and the patient vomited most of her food. The temperature reached 104° F. on the fifteenth day, and dullness, but no râles, developed at the base of the right lung. Sulfathiazole was given by mouth, but the patient did not retain it. On the following day sodium sulfapyridine was given intravenously. The report of the x-ray examination of the chest was: "Pneumonic type infiltration of mesial portion of right base." The patient felt pain at a well-circumscribed point at the base of the right lung; she was tapped here, and about 1 c.c. of heavy pus was obtained; it did not seem to contain any granules. On the following two days the patient was slightly more comfortable, the cough being nonproductive and persistent. The patient was tapped again on the nineteenth postoperative day at the point of greatest pain, and a few cubic centimeters of heavy creamy pus were obtained. The bacteriologic report on the smear and the culture of the pus both times was: gram-positive, micro-aerophilic, nonhemolytic streptococcus. The following day the patient looked cyanotic and toxic, coughed, and vomited continuously, and her temperature remained above 102° F.

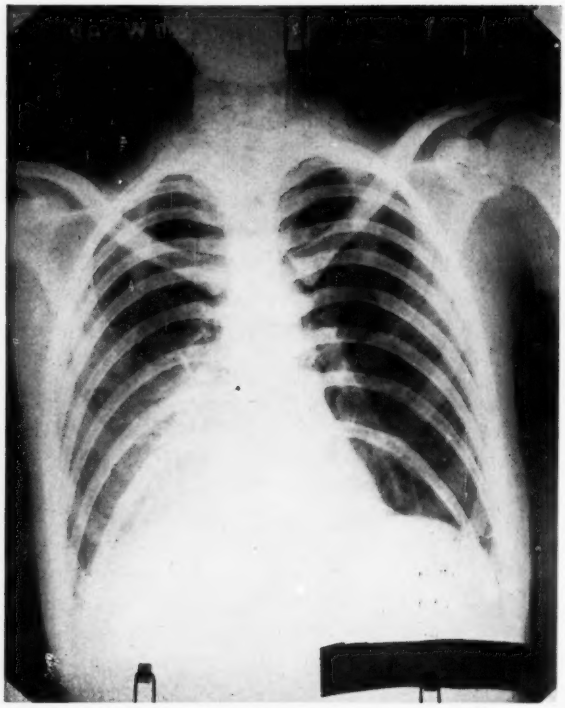

Fig. 1.—Roentgenogram taken on Feb. 26, 1941, showing pneumonic type infiltration of right lower portion of the lung.

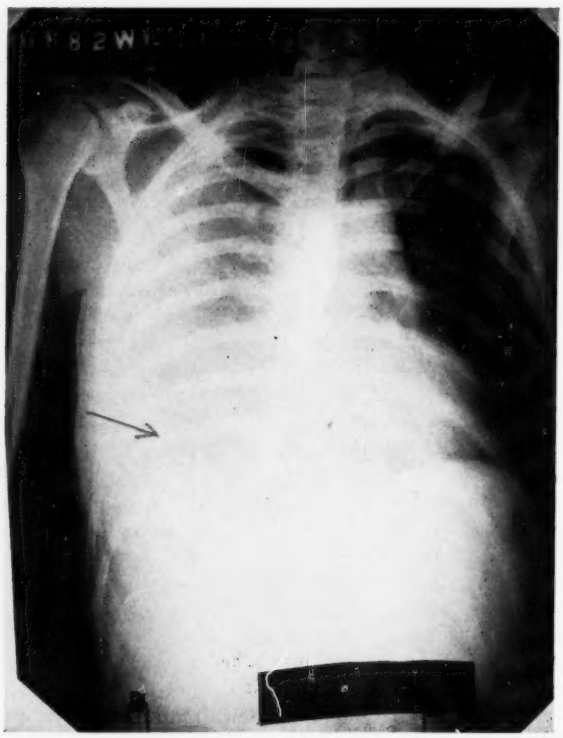

Fig. 2.—Roentgenogram taken on March 3, 1941, showing a marked spreading of process involving more than two-thirds of right lung area. Note seat of resection shown by arrow.

In view of these alarming symptoms coupled with the physical findings, and of having obtained pus twice by tap, it was felt that a diagnosis of empyema was justified. Dr. Aaron E. Parsonnet saw the patient in consultation. He concurred in this opinion and advised immediate thoracotomy. The patient was transfused and operated upon on the same day. A two-inch part of the ninth and tenth ribs was resected in the scapular line, and the pleural cavity was opened. It was found free of fluid or inflammatory changes; there were no adhesions between the two pleural leaves. The visceral pleura was yellowish and discolored over an area of about an inch. The lung was aspirated at that point, and a few cubic centimeters of creamy pus were obtained. A part of the lung the size of a silver dollar was sutured to the parietal pleura, and iodoform packing was placed in the wound. On the next day the lung was entered at the exposed part by cautery, one finger was inserted, and friable tissue was broken up. The cavity was drained by rubber tube and packed with activated zinc peroxide cream and gauze. None of the pus specimens nor the wound afterwards had a foul odor.

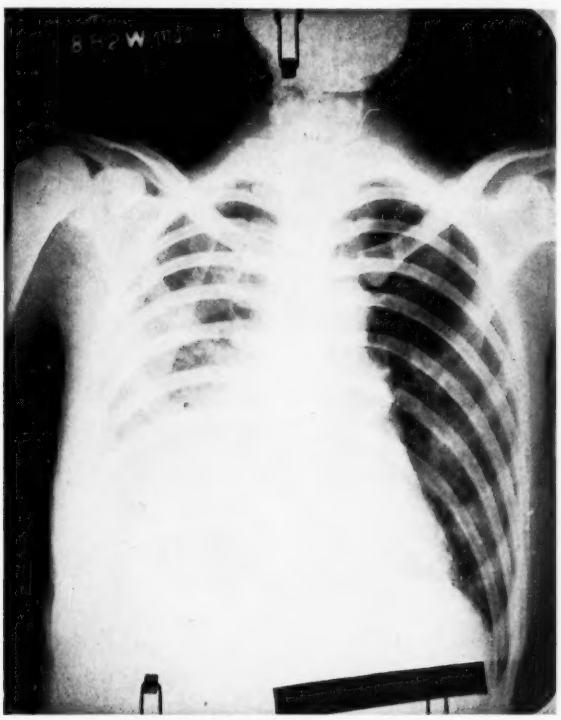

Fig. 3.—Roentgenogram taken one week later, March 10, 1941, showing marked recession of the process.

Within a few hours after pneumonotomy, an impressive change occurred. The general appearance of the patient improved strikingly, the cyanosis and toxicity lessened; never returning to the same degree during the eventful and rather stormy postoperative course.

The appearance of total empyema was feared; it did not occur. At the first change of the dressing, one day after the pneumonotomy, activated zinc peroxide cream (Z.P.O.) was injected through the drain tube into the lung cavity. It resulted in such an exasperating coughing spell, that its use had to be temporarily discontinued, and powdered sulfanilamide substituted for a while. Later Z.P.O. was used again. From the very first day of pneumonotomy a bronchial fistula was present. The temperature throughout the twenty days of hospitalization following pneumonotomy continued to be about 103° F. Jaundice was noticed on the ninth day postoperatively (bilirubin was 2.7 per 100 c.c.); it subsided as the general condition improved. The cough persisted; it was spastic, with very little sputum, and was often followed by vomiting. About two weeks after pneumonotomy, burrowing appeared at the

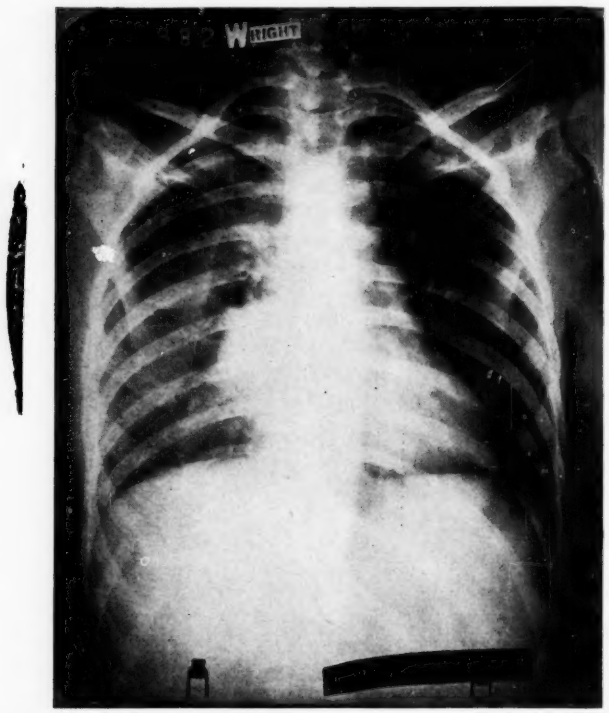

Fig. 4.—Roentgenogram taken on March 19, 1941. Note marked improvement and aeration of lung.

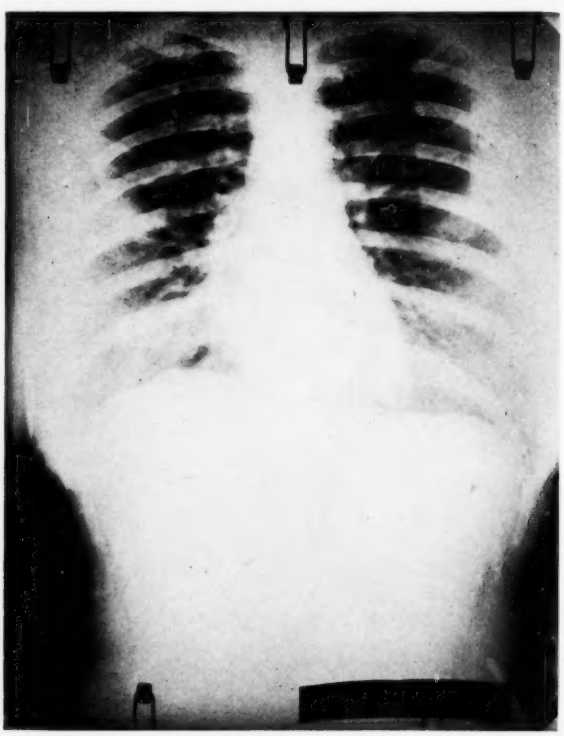

Fig. 5.—Roentgenogram of final plate on Sept. 9, 1941. Condition practically restored to normal.

upper and inner wound margins. Though the patient was cheerful, the persistent cough, the vomiting, and the high temperature caused further decline. The condition became stationary, and the patient was discharged on March 23, 1941, the forty-first day of hospitalization.

Up to April 15, the burrowing at the inner wound edge persisted, causing further loss of tissue. For a while Z.P.O. was used, followed later by azochloramid in triacetin. On April 13, the overhanging wound edge seemed to melt away at one point; it seemed that some superimposed infection helped to destroy it. By April 15 this melting-down process neatly scooped off all the overhanging edge. From this point on the patient's condition improved rapidly; the daily temperature curve, ranging between 102° and 102.6° F. daily, tapered down, and the vomiting ceased. Her weight, which came down to the dangerous level of 72 pounds, started to come back. The recovery became uneventful. The skin wound and the bronchial fistula closed completely by May 15.

Examination on September 9 found the patient weighing 114¼ pounds, and with no clinical symptoms. Of further interest is the fact that the patient was now completely relieved from right lower quadrant pain.

COMMENT

1. In this case the x-ray examination did not disclose an abscess. Jackson and Judd[5] reported that an abscess can be demonstrated only after drainage of some kind has been established, thus permitting the access of air into the abscess cavity. The alternative possibility of gas in the abscess would be gas formation by bacteria; such an occurrence, however, does not seem to have been reported in the literature. Palugyai[6] stated that "closed forms of abscess cannot be differentiated from tumor." Touroff and Neuhof[7] stated that acute abscess may show either cavity or dense infiltration. In this case, x-ray findings indicated a pneumonic type of infiltration.

2. There were no adhesions between the parietal and visceral leaves of the pleura, though a definite abscess was already present. This refutes the claim made by some that adhesions are always present. The abscess was not near an interlobar fissure, thus excluding the presence of invisible adhesions.

3. Pus was obtained twice, and later the abscess was found at the site of the most intense pain. This fact may be used as a lead in future cases.

4. The improvement within a few hours after draining the abscess was striking.

5. During recovery it was noted that the intercostal spaces were narrowed and that the right half of the chest, harboring the abscess originally, became smaller than the other half. Following complete closure of the wound, the chest re-expanded and the intercostal spaces widened.

6. Pus for culture is rarely obtained prior to the abscess opening into the bronchus. It cannot be obtained by bronchoscopy because the abscess did not perforate. It can hardly be obtained by operation because the examination of an abscess before perforation fails to disclose a cavity, and therefore, an operation is usually not performed. This, in large measure, is also the reason why aspiration is not attempted, the other one being the likelihood of a resultant empyema, following the needling of an abscess at an early stage. Superimposed infections easily occur after the abscess perforates, and therefore, examination will naturally disclose more than one bacterium. In the case reported, an aspiration was done because the physical findings indicated a pleural process. Both through error and lucky coincidence the needle entered the lung. Pus was found

twice, yet the pleural cavity was not infected. Thus the pus of a lung abscess was obtained under sterile conditions before the usual perforation would have occurred. The chief reason for only one bacterium being present may be due to the fact that there was complete absence of superimposed infection. It seems fair to assume that this bacterium caused the infection because the identical organism was obtained both times, and no other bacteria were found in either instance. The bacterium is a nonhemolytic or gamma streptococcus, belonging to the group that was reported by Cohen,[3] in each of his 16 cases. This bacterium, though micro-aerophilic, differs from the streptococcus described by Meleney; his being a hemolytic streptococcus not causing any persistent fever. Meleney and Harvey[8] stated that "there is usually a moderate fever and a moderate pain in the wound. At times the fever may reach 103° F. for a period of days, but usually it remains at a lower level." No cultures were made after pneumonotomy. It seems that this bacterium caused burrowing, high fever, and toxicity, while even the smallest amount of burrowing persisted, though secondary infection might have played a part in this. These features make this bacterium different from the Meleney streptococcus. At least one author, Reicher,[9] called attention to a streptococcus that was not hemolytic, but burrowing.

7. Finally, this streptococcus was the sole bacteriologic cause of a lung abscess.

SUMMARY

A case of lung abscess is reported. Pus for bacteriologic examination was obtained before the abscess perforated. The bacteriologic examination showed that only one bacterium was present, a micro-aerophilic, nonhemolytic, gram-positive streptococcus.

REFERENCES

1. Neuhof, H.: The Surgical Treatment of Acute Pulmonary Abscess, Dis. of Chest 7: 74, 1941.
2. Bucher, C. J.: Abscess of the Lung, A Bacteriological Study, Am. J. M. Sc. **179**: 406, 1930.
3. Cohen, J.: The Bacteriology of Abscesses of the Lung, Arch. Surg. **24**: 171, 1932.
4. Varney, P. L.: Bacterial Flora of the Treated and Untreated Abscess of the Lung, Arch. Surg. **19**: 1602, 1929.
5. Jackson, C. L., and Judd, A. R.: The Role of Bronchoscopy in the Treatment of Pulmonary Abscess, J. Thoracic Surg. **10**: 179, 1940.
6. Palugyai, J.: Die Roentgenuntersuchungen der Lungeneiterungen, Wien. klin. Wchnschr. **52**: 1137, 1940.
7. Touroff, A. S. W., and Neuhof, H.: Acute Putrid Abscess of the Lung, Surg., Gynec. & Obst. **68**: 687, 1939.
8. Meleney, F. L., and Harvey, H. D.: The Combined Use of Zinc Peroxide and Sulfanilamide in the Treatment of Chronic, Undermining, Burrowing Ulcers Due to the Micro-aerophilic, Hemolytic Streptococcus, Ann. Surg. **110**: 1067, 1939.
9. Reicher, J.: Superficial Infections Due to Anaerobic Streptococci. Indications for Zinc Peroxide Treatment, Surg., Gynec. & Obst. **72**: 651, 1941.

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BENZENE POISONING IN INDUSTRY

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AS THE national defense program alters industry to suit its needs, it is placing increasing burdens on the industrial physician. In the rubber industry one of the problems confronting the medical staff is the increasing use of benzol and the physical hazards it presents.

Pure benzene, C_6H_6, is a coal tar distillate. It is a colorless limpid liquid with a characteristic odor which is rather pleasant. It boils at 80.2° C. Its specific gravity is 0.879. It is soluble, 0.07 Gm. in 100 c.c. of water. It is somewhat soluble in alcohol. Pure benzene is not used in industry. The commercial varieties contain from 2 to 10 per cent toluene, xylene, olefins, paraffin, and carbon disulfide. It is an excellent solvent for rubber gums, resins, celluloid, and fats of all kinds. It is the best solvent for certain types of synthetic rubber because it dries faster and is cheaper. It is these properties that make its use so prevalent in the rubber industry today.

It is used extensively in the rubber industry, in the leather industry, in varnish and paint removing, in gilding and bronzing, in spray gun paints, coal tar paints, and to a small extent as a motor car fuel.

Benzol poisoning may result from absorption of the benzene by the respiratory tract or the alimentary tract, or through the skin. In the majority of cases absorption through the respiratory tract is the common mode of entrance. Absorption through the skin is slow. A considerable amount of fumes may act as an asphyxiation agent. With the use of animals the United States Public Health Service states that concentrations as low as 5 parts per million may cause symptoms. Cases of poisoning in human beings have been found with concentrations as low as 25 parts per million. Concentrations of 50 to 100 parts per million are considered to be safe for the average person. Individual susceptibility varies. Some are able to withstand much higher concentrations than others. This factor in itself causes a big problem in industry, since ventilation adequate to maintain a concentration within safe limits is still not enough to prevent the person whose tolerance is low from absorbing the chemical.

The following statistics are those of a factory in the rubber industry that has been using benzol in small quantities for years. With the advent of large war orders and the increasing use of synthetic rubber, patients came to the company hospital with complaints of malaise, nausea, and vomiting, and in a few cases, of bleeding. An immediate check was made on all employees using benzol to any extent. Complete blood counts were done on 1,104 people. Of this number 83, or 7.5 per cent, showed mild blood changes and gave symptoms of having some slight absorption of benzol fumes. Twenty-five, or 2.2 per cent, showed severe blood changes with symptoms of severe benzol intoxication. These patients had to be treated over a long period of time. Of the above 25 cases, 9 were confined to the hospital for a period of time and received blood transfusions

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Received for publication, March 12, 1942.

varying in number from a low of 2 to a high of 50. Three patients of the above 9 died. For absolute certainty in the diagnosis a bone marrow biopsy and study were done on all the severe cases. This degree of fatality of three patients out of 25 with aplastic anemia, or 12 per cent, is low. This low incidence was believed to be due to the extreme amount of treatment received. The treatment given will be explained later.

SYMPTOMS

Individual susceptibility is the governing factor in the appearance of symptoms. Some patients can apparently be exposed to large concentrations of benzol without effect. Others show symptoms when the concentration is as low as 5 parts per million. The United States Public Health Service states that young intelligent girls are the most susceptible.

Acute benzol poisoning is rare. The symptoms of an acute case are referable to the central nervous system. There are muscular tremors, salivation, violent twitchings, exhaustion, paralysis, narcosis, convulsions, and death from paralysis of the respiratory center.

The common cases are those of long exposure to fairly small concentrations of the fumes; that is, concentrations usually below 100 parts per million, with occasional sharp exposures of 500 to 1,000 parts per million, such as occur when fumes are obtained from the opening of a can of benzol or from the heating of material containing benzol. The concentration of fumes to which the patients studied were exposed varied between 50 and 500 parts per million, with the average concentration being about 100 parts per million. Then the symptoms are lassitude, malaise, nausea, vomiting, dizziness, and headaches, usually frontal in character. If there is any skin absorption, a macular dermatitis will appear at the site of exposure. As exposure continues the above symptoms become more severe. Petechiae, scattered over the body, appear. Hemorrhages under the skin and from the body cavities may occur. In one case ecchymoses and petechiae were the first symptoms that appeared. At the time of appearance the count was normal. In five days the white blood cell count was 500. Diplopia, especially for close distances, occurs in some cases. If the person is highly susceptible, or if the exposure has been great and prolonged, he may get physiologic or functional depression of the bone marrow. This varies in degree from a slight hematopoietic suppression to a total bone marrow aplasia.

The symptoms of a total aplasia are those of benzol absorption followed by prostration. On the other hand, it is sometimes strange how well the patient may appear. Aside from fatigue he may have no other complaints.

The best index of benzol absorption is the blood count. All blood counts should be done with pipettes that have been standardized by the United States Bureau of Standards. The improved Newcomer method of hemoglobin determination is preferable. All components of the blood are affected, being lowered. Most important, the total leucocyte count falls. Special reference should be made to the differential count. The ratio of polymorphonuclear cells to monocytes is changed. The number of monocytes increases and the polymorphonuclear cells decrease. The red blood cell count is lowered. If it makes a drop of 25 per cent, the employee should be removed from the fumes. The hemoglobin also falls. It has been reported by some investigators that the blood count

may be raised at first. However, when this occurs it is quickly followed by a general lowering of the count. The platelet count is lowered, dropping to practically zero in severe cases. The reticulocyte count is also lowered. The prothrombin time is normal. The resulting picture is one of aplastic anemia. One of our patients exhibited a count of 125 white blood cells, 1,500,000 red blood cells, and 25 per cent hemoglobin. This man died. The red blood cells exhibit anisocytosis, poikilocytosis, and polychromatophilia. In all patients showing a marked blood reduction a bone marrow biopsy should be done. The degree of aplasia can be determined, and usually an accurate prognosis can be made. Patients with total aplasia always die. Patients with partial aplasia have a prognosis dependent upon the amount of regeneration present.

Urine examinations are usually negative for albumin and sugar. The urinary sulfate test, which is a ratio between the total sulfates and the inorganic sulfates present in the urine, is useful in indicating absorption of fumes. The ratio is lowered when the absorption is great. This is because the inorganic sulfates are increased. This test is useful only at time of exposure, the ratio returning to normal in about four hours when the patient is in fresh air. Normally the ratio is about 0.75. In severe exposures it may be lowered to 0.25. Lowering of the ratio is an indication for removal of the employee from exposure to the fumes.

The blood pressure may be lowered. This test is not significant unless it is accompanied by other positive findings.

DIFFERENTIAL DIAGNOSIS

Aplastic anemia is to be differentiated from agranulocytosis and acute leucemia. The symptoms of both of these diseases are very similar. In agranulocytosis the red blood cell and platelet counts are usually essentially normal. In acute leucemia the white blood cell count may be normal, raised, or lowered. The differential count shows a marked increase in very young white blood cells at the height of the disease of acute leucemia. The liver and spleen are usually enlarged in acute leucemia and are normal in aplastic anemia and agranulocytosis. The lymph nodes are normal in aplastic anemia and are usually enlarged in leucemia. In agranulocytosis the lymph nodes of the neck are usually enlarged and are painful and tender. Hemorrhages are common in aplastic anemia and leucemia, and are rare in agranulocytosis. Sore throat is common in leucemia and especially so in agranulocytosis. It must be recognized that the differential diagnosis between these three diseases may be difficult. Bone marrow studies usually reveal the proper diagnosis.

PROPHYLAXIS

This problem is one of major importance. The cooperation of the management of the company is necessary. Many different ideas have been expressed on the subject. Of course, the ideal situation would be to do away with the use of benzol. Toluene is mentioned as a substitute. This chemical, which is methyl benzene, is not as toxic as benzol because of its higher volatility. However, sufficient concentrations of the fumes of toluene will act in the same manner as benzene.

Adequate ventilation is an important prophylactic measure. A closed system of ventilation where the workman comes in contact with no fumes is ideal. Any other type of ventilation is not entirely safe. So long as any fumes are present it is possible that intoxication may occur.

The health of people working in benzol should be kept under constant supervision. Any illness or symptoms of illness should be investigated. Frequent blood counts should be made. Occasional urinary sulfate tests are of value.

The following program is suggested:

1. The testing of frequent air samples with a combustible gas indicator. Introduce ventilation in an endeavor to keep the concentration of benzol in the air as far below 100 parts per million as possible.
2. All new employees, before being permitted to work, should be given a complete blood count. Any persons with blood dyscrasias should not be permitted to work in benzol departments. No one should be allowed to work in a benzol department with a white blood cell count below 5,000, or above 13,000.
3. After ten days' employment the employee should be given a urinary sulfate test at the end of a working day. If the urinary sulfate ratio is below 50, there is a tendency to absorption, and the employee should be removed from the benzol department.
4. Any employee exhibiting symptoms should be sent to the hospital for blood studies and physical examination. A drop of 25 per cent in any blood components is sufficient reason for removal. An employee who is so removed should be given one week vacation and then placed on another job. During the week off the employee should remain as much as possible in the open air. At the end of the week the blood should be rechecked. If normal, the employee may be permitted to return to work in a nonbenzol department.

An ideal arrangement would be to use a system of repeated blood checks on exposed employees. If this were done at intervals of approximately every two weeks, it would be of benefit, but even then no positive guarantee could be given that poisoning would not occur. This type of test may be prohibitive in large industry because of the number of technicians needed to perform the counts.

TREATMENT

In patients with mild intoxication, with or without a drop in the blood count, the treatment is removal from the fumes and plenty of fresh air. These patients will return to normal in a few days with no medication.

In patients in whom absorption is sufficient to cause a marked change in the blood picture more active treatment is necessary. These patients are placed at absolute bed rest. Multiple small whole blood transfusions (about 250 c.c.) are given. One patient was given 50 transfusions, another 36. The patient is alkalinized with sodium or potassium citrate on the possibility that this might prevent a reaction to the transfusions due to acidosis. When severe reactions occur, blood plasma may be used in place of whole blood.

Direct bone marrow transfusions may be given as often as twice weekly. In this procedure 2 to 5 c.c. of bone marrow are removed from the sternum of a compatible donor and introduced directly into the sternum of the patient. From the same donor 250 to 300 c.c. of blood are drawn and transfused into the patient. This is followed by 1,000 c.c. of normal saline solution.

Ten milligrams of liver are given intramuscularly daily. Large daily doses of liver, iron, calcium, phosphorus, yellow bone marrow, and multiple vitamins are given by mouth. A total of 400 to 600 mg. of ascorbic acid are given daily by mouth. This has been claimed by some investigators to be a necessary adjunct in treatment. A full diet is prescribed. The patient's general hygiene is improved as much as possible. Bleeding areas are stopped if at all possible. Pentnucleotide has been used and found to be effective in some cases.

The resistance of these patients is low due to the agranulocytosis. Secondary infection, the most common of which is a Vincent's organism infection of the mouth, is one of the causes of death. For certain types of severe secondary infection sulfadiazine in 4 Gm. daily doses has been used. Sulfanilamide powder was used on one case of secondary streptococcus infection with good effect. However, the sulfone products must be used with care since they are bone marrow depressants.

The response to therapy is slow since a considerable time is necessary for the bone marrow to regenerate. One patient has been under treatment for twelve months. He still has a low hemoglobin and red blood cell count. It has been suggested that a splenectomy be done on patients who do not respond to therapy, the rationale being that the spleen has an inhibiting effect on the bone marrow. This procedure has been reported to be successful in several patients who otherwise would probably have died.

The treatment at best is not wholly satisfactory. In too many patients the disease proves fatal. The problem of the use of benzol has become a large and vital one and as yet is not solved.

Probably the most important fact to remember about the use of benzol for industry is never to sacrifice caution in the use of benzol because the result of carelessness with this chemical is usually death.

SUMMARY

Out of 1,104 people tested who were exposed to concentrations of benzol fumes averaging about 100 parts per million for varying periods of time, 83, or 7.5 per cent, show slight blood changes, indicating slight absorption of the fumes. Twenty-five, or 2.2 per cent, showed marked blood changes. Nine of these 25 patients had a severe enough aplastic anemia to demand hospitalization. Three patients died, a 12 per cent fatality out of all the patients showing absorption, or 0.27 per cent fatality out of all the people exposed to the fumes. This degree of fatality of patients with aplastic anemia is considered to be low. The form of treatment was multiple whole blood and direct bone marrow transfusions as well as liver, iron, calcium, phosphorus, yellow bone marrow, and multiple vitamin therapy.

Prophylactic measures recommended are adequate ventilation and constant medical supervision, which is best done by frequent blood examinations.

THE BLOOD PATTERN AS A CLUE TO THE DIAGNOSIS OF MALIGNANT DISEASE

H. Leonard Bolen, M.D., Fall River, Mass.

THE early diagnosis of carcinoma is a goal eagerly sought by science in order that treatment may be undertaken before it is too late. The greatest obstacle to progress in cancer therapy is the vagueness of the early complaints and the obscurity of physical findings. As Fitz remarked in 1939:[1]

"Cancer presents a bewildering problem. To me the impressive feature of watching such cases was the apparent suddenness of onset of important symptoms. . . . If this is true, what is badly needed is a test for carcinoma analogous to the tuberculin test for tuberculosis and the Wassermann test for syphilis, so that cancer can be recognized in its preclinical state. Until such a test is discovered, cancer inevitably must continue to be discovered in most cases, when it is already far advanced."

To this end investigators have utilized the laboratory in an effort to discover the presence of cancer cells in the body before the appearance of clinical symptoms and signs. In 1940 these statements appeared[2] regarding the status of the search for a serologic test for cancer:

"It may be doubted whether it will be possible for science ever to place at our disposal a test so delicate as to indicate the existence of a few cancer cells in the human body; yet this is precisely the type of test most desired."

"Ordinary examination of the blood reveals no condition peculiar to malignant disease. Most observers have found a decrease in red cells, some have found a diminution in hemoglobin, others have noticed an increase in leukocytes. All these findings, however, are present only in the later stages of the disease. In many cases reported, the notation *blood negative* will be found."

According to Stahl,[3] the origin of blood and the origin of cancer are very closely interwoven in primal embryologic study. Investigation indicates that there is a specific cancer cellule. This is always an immature, never a mature, cell. In the normal organism a growth-control hormone is present; when this ceases to function, the cancer cells grow, and if this growth is not checked, the neoplasm progresses, causing devastation and death.

In 1935 Boyd[4] stated that cancer may be regarded in two ways. It may be the expression of a power for unlimited proliferation, which develops in a tissue at a certain age in its genetic life history; or a malignant condition may be imposed on cells from without by a number of carcinogenic agents, such as coal tar or dibenzanthracene. Of the exact mechanism nothing is known, and may never be known.

Received for publication, March 13, 1942.

Does the answer lie in a study of the blood? In a physiologic sense the blood is a part of every tissue, and any change involving the fixed tissues of the body must affect the blood. Thus we have a picture of deformity of the red blood cells in anemias sufficient to clinch the diagnosis even without clinical evidence. Hematopoiesis is the sum total of the integrated functioning of all the tissues of the body.

There is not space to review the various tests which have been proposed to detect the presence of cancer cells in the human body. Fuchs's[5] test for malignancy appeared in 1925. He examined the blood of 92 persons with cancer and 48 controls without malignant disease. The blood serum from every cancer patient digested heated human fibrin; the blood serum of the controls was invariably negative. This test has furnished the basis for later investigation. Recently Robinson[6] used a modification of the test in 200 patients in the Vanderbilt University Hospital. Rosenthal[7] published results with this method at the University of Pennsylvania. Esculies of Buenos Aires[8] discussed results in 1940.

They stated that they isolated an ether-soluble protein from the serum of cancer patients, which was either the product of abnormal metabolism, or a protective ferment called into being against the invasion of abnormally metabolized protein derivatives. Further investigation is to be carried on to determine whether the protein isolated by these authors possesses fermenting activity in the presence of the same serum from which it was extracted, as well as the precise polarimetric deviation.

This test has the following limitations:

1. The growth must be over six months old.

2. The patient should not have been operated upon or have received radium or roentgen-ray treatment for at least a month.

3. The tumor must be in a period of active growth.

Pfeiffer's[9] crystallization method for the diagnosis of cancer, which appeared in 1938, has also been widely used. He claimed that the patterns which crystals assumed after the addition of diluted blood to a solution of copper chloride was a method of differentiating between health and disease. A certain cross formation was characteristic of tuberculosis; a wing pattern was indicative of cancer. In cancer, however, the blood picture is decentralized and disorganized. The more numerous and defined the groupings, the more advanced the cancer. The fact that cancerous blood actually produces these differences, which are uniform in kind, is readily established even with a few tests. Rascher of Munich[10] and Gruner of Montreal[11] have used Pfeiffer's method with personal modifications and have obtained a high degree of accuracy in diagnosing cancer.

The test is fairly simple. One drop of blood from a finger prick is allowed to fall into 1 c.c. of distilled water; after thirty minutes 2 drops of this are introduced into 10 c.c. of a 20 per cent solution of copper chloride. After thirty seconds the mixture is poured out upon specially prepared plates resting in a chamber. Readings of the crystallizations are made after eighteen hours.

Gruner uses polarized light to read the slides. He secured positive results in 110 of 122 patients, and in 33 normal persons the test was positive only once. Rascher examined 246 persons. In 98 there were no signs of cancer, and the clinical picture agreed in every case. In 148 patients the plates indicated cancer. In 11 the diagnosis was incorrect, three being normal, and eight having sarcoma instead of carcinoma. In nine the clinical diagnosis of cancer was doubtful. In 128 the plates were correct; all these patients had carcinoma.

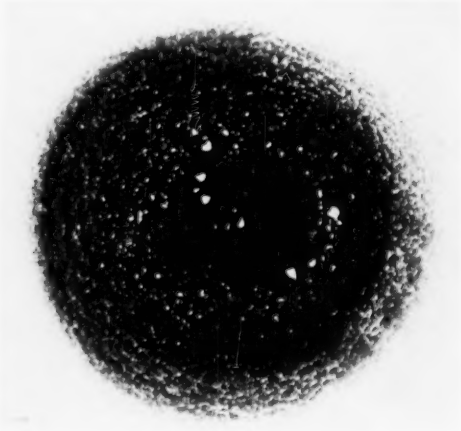

Fig. 1.

For this test the technique must be extremely exact, and only fresh blood can be used (not later than five hours old). In some instances the test was repeated after excision of the cancer. In some cases the test became negative, which was interpreted as indicating complete removal. If the test was repeatedly positive, the patient was watched carefully, even though no tumor could be located.

Reports from the Lankenau Hospital[12] show an 80 to 85 per cent of reliability in diagnosing early cancer. The test needs simplification and is available only in a large medical center because of the difficult technical procedures. The cancer pattern is described as two wings rising from a straight basis line, both wings extending on the same side of the basis line.

The blood sedimentation rate has been employed for many years as a diagnostic aid. Its use in gynecology, obstetrics, and urology, and the different methods of performing this test are set forth in an excellent article by Hirsh.[13] He outlines the Schiller modification of this test and its wider application, and analyzes the various theories regarding the factors which influence the speed with which the erythrocytes settle. The most probable explanation is that toxin production involves a breaking down of tissue proteins, and the products of disintegrated tissue proteins stimulate fibrinogen formation. The following statement of Hirsh is significant:

"We believe the sedimentation rate depends on the presence of a foreign protein in the circulation. In pregnancy, this is the protein of fetal catabolism;

Fig. 2.

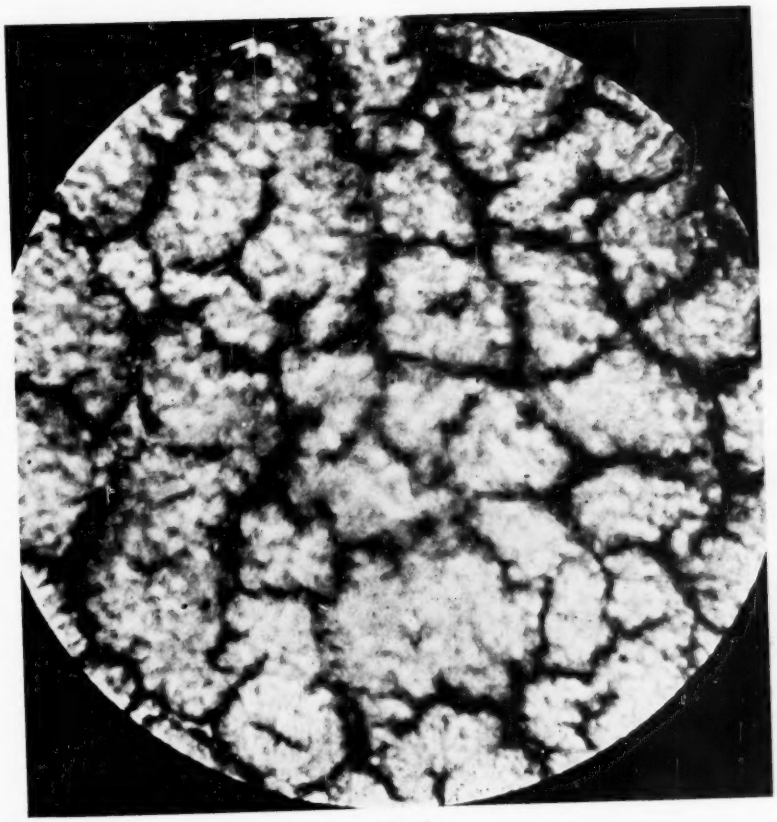

Fig. 3.

in infection, it is the protein of bacteria, and in malignancy or coronary occlusion, it is the protein of necrotic tissue."

The test, although not specific for any one condition, is of great value in estimating the degree and progress of disease. According to Schiller:

"If a malignant growth be removed operatively, the blood sedimentation rate should return to normal within six weeks if complete removal has been obtained. If the rate returns to normal and remains normal at least six months, one may give a guardedly favorable prognosis. If the rate becomes abnormal within this time, it is suggestive of local recurrence of the growth or metastasis."

Wolfson and his co-workers[14] recently published an article on early diagnosis of malignant metastases to the spine, and discussed the value of the erythrocyte sedimentation rate, which has a definite correlation with metastatic involvement of bone. Malignancy is often associated with an elevated phosphatase level and a rapid sedimentation rate.

In 1939 Goldberger[15] published his report of a rapid bedside test for measuring the blood sedimentation rate. He pricked the finger of the patient and allowed a small drop of blood (about 3 mm. in diameter) to touch the undersurface of a clean glass slide. Since the first drop collected will be thickly rimmed, a second and third drop are touched to the slide. Then the slide is righted and the drops of blood are allowed to clot and dry spontaneously in a horizontal position. When dry, the blood is observed macroscopically by holding the slide up to the light. The criteria for reading the film are as follows:

1. Fineness or coarseness of general detail.
2. Character of meshwork, if present.
3. Presence or absence of central agglutination.
4. Presence or absence of peripheral rings.

With these in mind, four classifications of sedimentation rate can be made:

1. Normal—very fine detail; no meshwork; gradual transition from periphery to dark central agglutinated mass.
2. Moderately rapid—detail somewhat coarse, but uniform throughout; meshwork not particularly noticeable; there may be the beginning of a central agglutinated mass.
3. Rapid—coarse detail; meshwork fine, but easily observed; no central agglutinated mass.
4. Very rapid—very coarse detail; definite meshwork; no central agglutinated mass.

By careful study of a great number of slides, Goldberger found that this method proved to be a simple, rapid, qualitative gauge in the diagnosis of coronary thrombosis, rheumatoid arthritis, salpingitis, etc. He found that the rate at which the red blood cells settle is directly proportional to the degree of their rouleau formation. Moreover, there was no correlation between the red blood count and the character of the drop of blood on the slide.

This problem of the blood sedimentation continues to give rise to much speculation. Rubin and Smith[16] believe that the volume of the erythrocytes

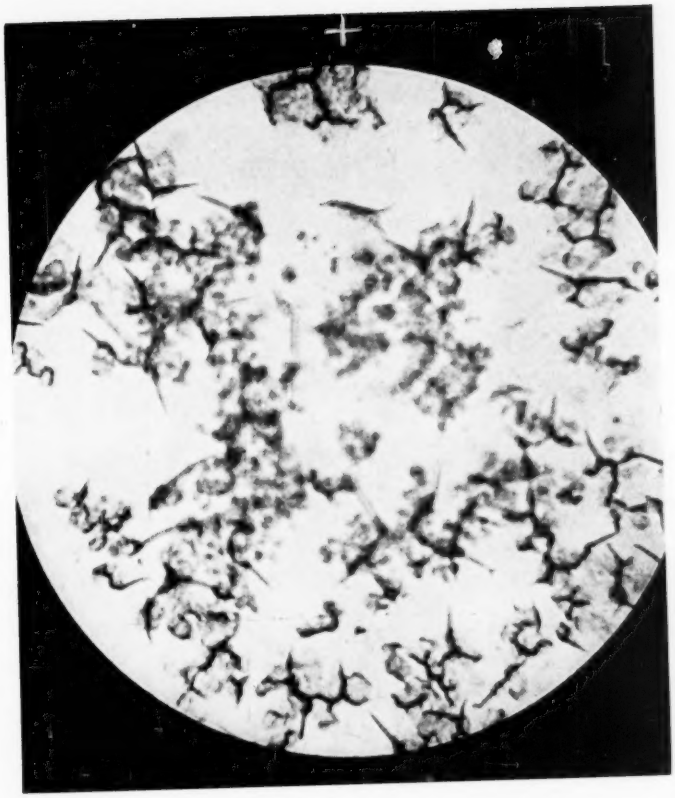

Fig. 4.

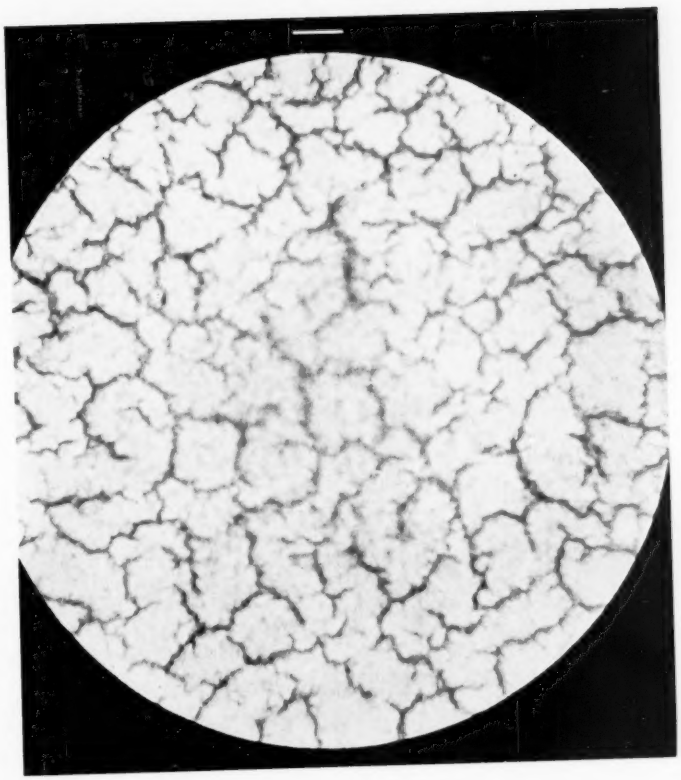

Fig. 5.

exerts an important influence on the sedimentation reaction under all conditions, and that probably physiologic changes in the sedimentation rate are also, to a great extent, influenced by the erythrocyte factor.

Davison,[17] in studying the separation of the blood cells from plasma (sedimentation), concludes that the process is dependent on rouleau formation. In normal or slowly sedimenting blood, each rouleau contains few erythrocytes, and the cell aggregates appear evenly distributed. In pathologic blood showing rapid sedimentation, each rouleau contains many corpuscles, and the rouleaux are clustered together, leaving large areas of free plasma between them. The sedimentation rate is directly proportional to the size of the cell aggregates or rouleaux.

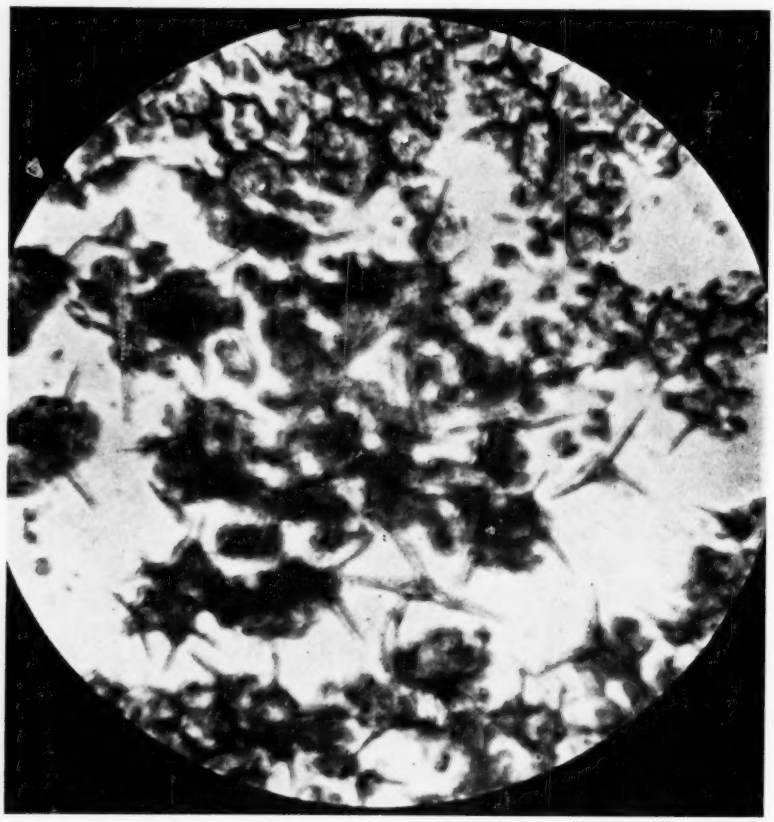

Fig. 6.

Impressed with the simplicity of Goldberger's method, the author decided to obtain a similar record of the blood on every patient, to see whether it would afford material aid in diagnosis, prognosis, and therapy. In each case, the finger was pricked, and 3 small drops of blood were collected on a glass slide and allowed to dry after the method of Goldberger. The patterns which the blood assumed, both macroscopically and microscopically, have proved of great value, especially when considered with clinical and laboratory findings.

The most interesting phenomenon was the distinctive pattern of the blood obtained from cancer patients. The pattern characteristic of normal blood

and of blood from patients with ulcer, inflammatory disease, etc., is devoid of meshwork. There is no breaking up of the fibrin; the blood is more compact. In the central part of each drop of blood is a dark agglutinated mass (Fig. 1). Invariably, the pattern formed by cancerous blood is that which I describe as the "dotted curtain" type (Fig. 2). Peripheral rings are absent, and no agglutinated mass can be seen in the center. The blood is broken down into "dots." The microphotograph of normal blood (Fig. 3) and (Fig. 5) is contrasted with the microphotograph of cancerous blood (Fig. 4) and (Fig. 6). Under the microscope the cancerous blood shows numerous three-cornered, tri-asteroid spicules scattered throughout. In no other condition have these spicules or rays been found in such abundance.

In six conditions listed in Table I I have demonstrated that a positive pattern was obtained. In all these, however, the clinical findings have differentiated the condition from cancer. Moreover, the positive reaction gradually changed to negative as the proper treatment was instituted. The laboratory analysis of the blood shed no light on the problem of diagnosing cancer.

TABLE I

CONDITIONS YIELDING TEMPORARY CARCINOMA PATTERN

	POSITIVE	NEGATIVE	TOTAL
Acute rheumatic fever	3	7	10
Rheumatoid arthritis	2	2	4
Active tuberculosis	5	8	13
Pregnancy	3	4	7
Pernicious anemia	2	2	4
Coronary disease	4	6	10
Total	19	29	48

TABLE II

CASES OF APPARENTLY CURED CANCER

	POSITIVE	NEGATIVE	TOTAL
Clinically Cured After Surgery:			
Breast	0	2	2
Stomach	0	1	1
Rectum	1	3	4
Prostate gland		2	3
Clinically Cured After Irradiation or Radium Therapy:			
Epithelioma, face	0	1	1
Hodgkin's disease	0	1	1
Breast	1	3	4
Sarcoma	0	1	1
Total	3	14	17

The same transition from positive to negative blood pattern on the slide occurred when "cures" were obtained in cancer patients, either by total extirpation surgically of the neoplasm, or radium implantation, or deep x-ray therapy.

The cases I have had in this category are noted in Table II.

Although the cases listed in Table II are few, the tendency is for the blood pattern to approach normal with eradication of cancerous elements. In the great majority of cancer cases, whether treated or untreated, the blood shows the

typical carcinoma pattern described above. It is so characteristic that it is usually possible to identify at a glance which of the slides bear the cancer pattern merely by holding them up to the light.

No relation has been found between the blood count and the carcinoma pattern. The similarity of the blood analyses in normal and cancer patients is apparent from a study of 50 unselected cases in each category, as seen in Table III.

TABLE III

TYPE	NO. OF CASES	HEMOGLOBIN	ERYTHROCYTE COUNT	LEUCOCYTE COUNT
Normal	50	70%-92%	3,200,000 to 4,500,000	7,500 to 10,000
Carcinoma	50	40%-90%	3,000,000 to 4,200,000	5,900 to 18,000

Tables IV and VI include analyses of the various conditions encountered in my practice since 1939, and the type of blood patterns which were noted on the slides. In some instances, these tests were made on the same patient weekly or monthly, before and after treatment for the specific condition.

TABLE IV

GROUP OF NONCANCER CASES

DISEASE	POSITIVE	NEGATIVE	TOTAL	% POSITIVE
Appendicitis	0	10	10	0
Diverticulitis	1	4	5	20
Encephalitis	0	1	1	0
Endometriosis	1	1	2	50
Epilepsy	0	6	6	0
Gall bladder disease	0	28	28	0
Hypertension	0	15	15	0
Meningitis, tuberculous	0	2	2	0
Pneumonias	0	5	5	0
Poisoning (methyl alcohol)	0	2	2	0
Tuberculosis (arrested)	0	4	4	0
Ulcer, gastric and duodenal	0	34	34	0
Ulcerative colitis	0	12	12	0
Miscellaneous	0	6	6	0
Total	2	130	132	1.5

Thus in this group of noncancer cases selected at random, there was a positive reaction in the blood in two conditions only; the margin of error was only 1.5 per cent. All readings in cases of appendicitis were normal regardless of the pathology. A case of ruptured appendix with peritonitis, myocardial damage, hypertension, diabetes mellitus, and a high nonprotein nitrogen reading showed a negative blood pattern. There were no positive readings in the gastric and duodenal ulcer patients, even when the hemoglobin was lower than 65 per cent. In the arrested stage of tuberculosis a normal pattern was seen, although a positive pattern was obtained in several patients with active tuberculosis. Also, in cases of coronary occlusion a positive pattern was noted about the second or third day. There was a return to normal as the condition of the patients improved.

Table V contains a classified list of cases of proved cancer. The slides of 140 of these patients were reviewed. In this series the age incidence is indicated.

TABLE V

AGE GROUP	SEX		TOTAL NUMBER OF CASES
	MALE	FEMALE	
Under 40 years	5	4	9
40 to 50 years	5	12	17
50 to 60 years	19	16	35
60 to 70 years	23	18	41
Over 70 years	17	21	38
Total	69	71	140

The results of the blood test for cancer are tabulated in Table VI.

TABLE VI

PROVED CARCINOMA CASES

SITE	POSITIVE	NEGATIVE	NUMBER OF CASES
Adrenal glands	1	0	1
Bladder	3	1	4
Breast	9	0	9
Breast, recurrent	3	0	3
Breast, metastases to lung	4	0	4
Breast, skeletal metastases	6	0	6
Gastrointestinal tract:			
Esophagus	1	0	1
Stomach	35	2	37
Colon	27	0	27
Rectum	11	0	11
Epidermoid carcinoma, cheek	1	0	1
Epidermoid carcinoma, nose	1	0	1
Throat	1	2	3
Larynx	1	0	1
Skin	1	0	1
Pancreas	4	0	4
Prostate gland	5	1	6
Uterus	7	2	9
General carcinomatosis	1	1	2
Hodgkin's disease	2	1	3
Sarcoma, osteogenic	0	2	2
Miscellaneous	4	0	4
Total	128	12	140, or 91.4% positive

A comparison of the noncancer and cancer cases revealed that a positive pattern was often obtained when there was no sign of anemia. The red blood cell count was an unreliable guide as to the state of the patient and the stage of the disease. With decrease in the cell count there was a corresponding decrease in the hemoglobin. A leucopenia was noted in patients with skeletal metastases. Increase in the white blood cell count invariably denoted peritoneal involvement or infection. But the red blood cell count, the white blood cell count, and the hemoglobin were of no help in differentiating between cancer and noncancer cases.

Two representative cases are herewith reported in some detail to illustrate the value of the test in diagnosis and treatment.

CASE 1.—J. C., a white male, aged 47 years, was seen at the office on January 6, 1940. At this time he stated that he had always been well until about two years before. In January, 1938, he began to have attacks of mid-epigastric pain, which occurred an hour after meals. These were relieved, at first, by alkalies or by vomiting. Attacks increased in frequency and severity. In February, 1939, he was x-rayed and told that he had a "growth" in the stomach. He was put on a diet and given "injections."

He continued on this regime for another year. Heartburn, postprandial pain, and vomiting occurred at frequent intervals, and the patient became weaker. He had a good appetite but was afraid to eat because of the subsequent pain and distress. He lost 30 pounds and began to complain of dyspnea on exertion.

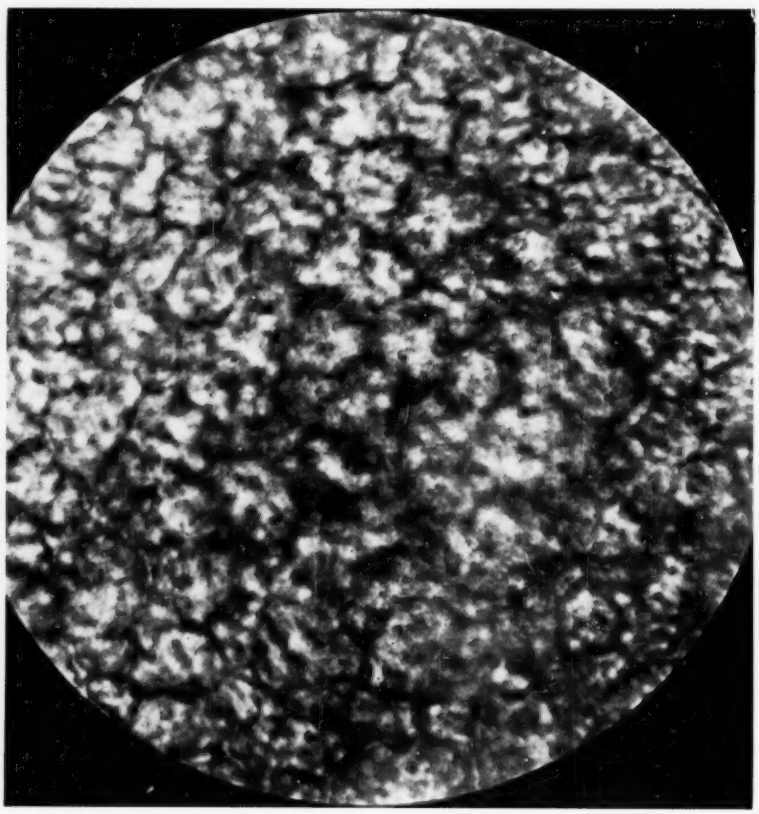

Fig. 7.

When first seen at our office in January, 1940, he stated that the pain had increased in severity. However, it never radiated to back or shoulders. He had no cough, hemoptysis, hematemesis, or melena. He was thin, pale, and listless. There was slight tenderness in the epigastric region, but no palpable mass. Three drops of blood on the slide showed a normal pattern. The patient was placed on a Sippy regimen. The following month the blood test was repeated and showed no change.

Because of inability to retain food and continual pain, the patient was seen again at the office on March 10. Another slide of the blood was made. In this the central nucleus was absent and there was definite formation of spicules. Since I felt that he had gastric cancer, patient was immediately referred to the hospital for surgery.

X-ray examination of the gastrointestinal tract was made. The impression of the radiologist was gastric ulcer, prepyloric.

Examination of the blood showed 44 per cent hemoglobin, 3,630,000 erythrocytes, and 8,400 leucocytes. The differential count was normal. There was very slight variation

in the size and shape of the red blood cells. The blood sugar was 95 mg., the nonprotein nitrogen was 33 mg. The benzidine test for occult blood in the stool was positive.

Gastric analysis on March 11, 1940, showed the following:

		Free Acid	*Total Acidity*
Fasting	68 c.c.	None	8 degrees
1st specimen	3 c.c.	10 degrees	32.5 degrees
2nd specimen diluted with water		10 degrees	16 degrees
3rd specimen	10 c.c.	20 degrees	80 degrees
4th specimen diluted with water		12 degrees	29 degrees
		52 degrees	165.5 degrees

On March 15, 1940, a subtotal gastrectomy was done for what appeared to be a prepyloric ulcer. A regional node was also excised for pathologic examination. The pathologic report, to the surprise of radiologist and surgeon, was gastric ulcer with small focus of carcinoma and metastasis to the regional lymph nodes. Fig. 8 shows a portion of the stomach with neoplasm removed at operation.

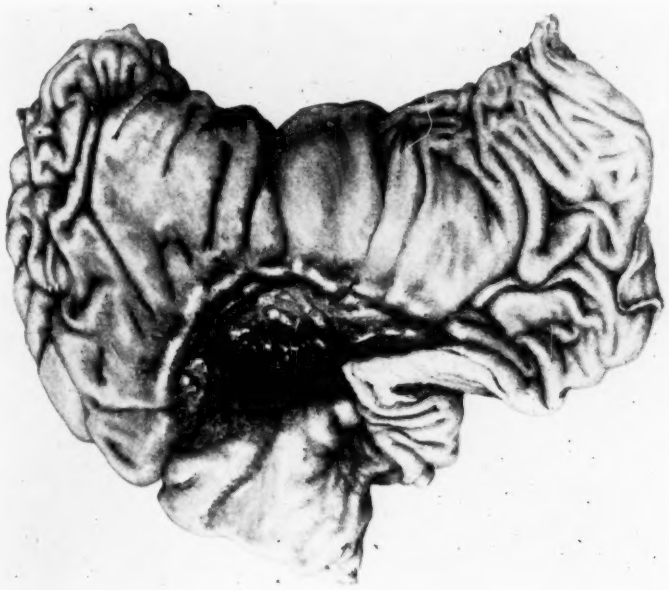

Fig. 8.

Except for an upper respiratory infection on the fifth to eighth postoperative days, the patient made a satisfactory convalescence, and was discharged improved April 7, 1940.

Six weeks after operation he was seen at the office. Examination of the blood showed that the pattern on the slide was now that of normal blood. This patient has been seen at frequent intervals since operation. There has been no recurrence of symptoms clinically. He works every day and has gained over 60 pounds. To date, January 15, 1942, the blood pattern on the slides has remained normal.

CASE 2.—D. P., a housewife, 46 years old, was admitted to the General Hospital on January 1, 1941, with the chief complaint of pain in the lower abdomen above the pubis, more severe after the menses. She stated that for the last three months menstrual pain had started about three days before the onset of flow, continued throughout the menstrual period of six or seven days, and persisted for a week after cessation. There was also a watery pinkish discharge for three days following menstruation.

She reported no previous serious illness. Catamenia began at the age of 15, a regular twenty-eight-day cycle, heavy flow, of nine days' duration. She had borne six children, all normal deliveries.

The patient's mother died of cancer of the uterus. One sister had uterine fibroids. There was no family history of tuberculosis, heart disease, allergy, or diabetes mellitus.

Physical examination revealed tenderness in both tubo-ovarian regions and in the suprapubic area. No masses or spasm were noted. The cervix was low near the introitus, large, both lips somewhat firmer than normal, both containing several small cysts. There was slight cystocele and rectocele.

Examination of the blood revealed 70 per cent hemoglobin, 4,100,000 erythrocytes, and 13,000 leucocytes. The differential count was normal. Three drops of blood on the slide, however, showed definitely the carcinoma pattern.

The clinical diagnosis was uterine fibroids.

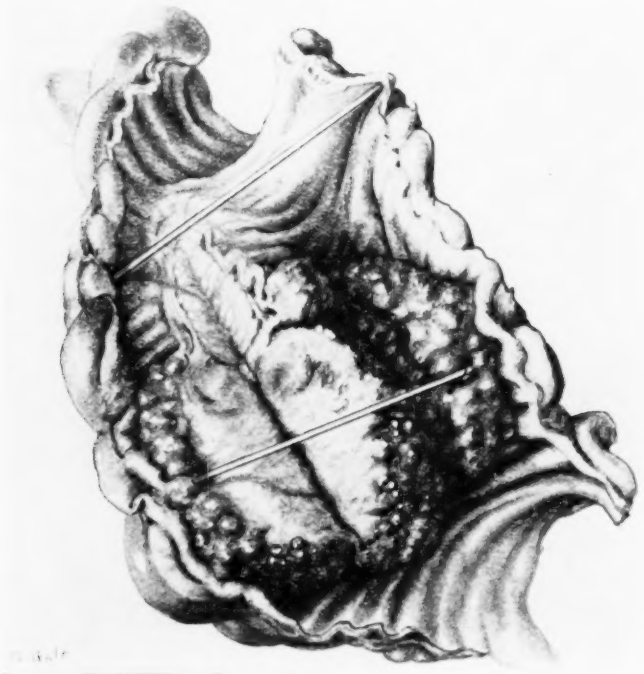

Fig. 9.

Operation was done on the following day, January 2, 1941. Laparotomy revealed a large tumor mass attached to the sigmoid. The tumor and the adjacent mass were brought out through the incision, and the wound was closed around them. On the ninth day the exteriorized loop of bowel and the tumor were removed (Fig. 9). The pathologic report was as follows:

The segment of sigmoid colon removed measured 18 by 8.5 cm. Protruding into the lumen and causing constriction, but not complete obstruction, was a large cauliflower growth, measuring 8 by 6 by 4 cm. Microscopically, the tumor mass was composed of columnar, glandular, epithelium; several mitotic figures were noted. The tumor invaded the submucosa. Diagnosis: Adenocarcinoma of the sigmoid.

This patient remained in the hospital. On March 15, 1941, rectal examination revealed metastases to the rectum. The patient was discharged on June 28, 1941. She is still alive at home, complaining of pain in the region of the sigmoid and rectum.

CONCLUSION

After a review of over 4,000 of these slides, two dominant blood patterns were noted: the first characterizes that of healthy persons and patients with

a variety of complaints; the second characterizes that of patients with cancer, and also those who have primary pernicious anemia, some with rheumatoid arthritis, and a few in late pregnancy. When treatment was instituted, however, in all but the cancer patients, the blood pattern changed to normal. In the cancer patients the typical "dotted-curtain" pattern persisted, except when total extirpation was achieved. Slides made on cancer patients up to within half an hour before death showed the same honeycombed appearance of the blood pattern. In patients in whom surgery had apparently removed all the tumor, and in some patients treated by radium or deep x-ray therapy, the blood pattern was found to return to normal after an interval of six weeks or more. No blood slides on patients who have remained well after operation for cancer have been made sooner than six weeks after operation. In one case of "cured" cancer of the stomach, the patient has had slides made weekly for a period of twenty-three months, and these have all been normal.

The erythrocyte count, the leucocyte count, and the hemoglobin appear to have no relationship with the carcinoma blood pattern on the slide. In my experience in cancer patients the blood picture changes relatively late. There is often a high red blood cell count and hemoglobin, with no indication of a malignant process. The changes in the blood picture occur only after clinical symptoms and signs have made it apparent that the disease is well advanced. Therefore, I have found this blood pattern test on the slide, when employed routinely, is of great value in suggesting, at least, the presence of carcinoma before it is discovered clinically or by roentgen ray.

Constant familiarity with a long series of slides enables the examiner to obtain a high percentage of correct readings. Erroneous interpretations have resulted, but the margin of error is small. This has happened when the technique has been faulty and the drops of blood have been too thick or have been smeared or not allowed to dry sufficiently; also the blood picture has changed somewhat when patients have had deep x-ray or radium therapy or repeated transfusions. We have already referred to those patients with pernicious anemia, etc. (Table I) in whom the reaction has been positive for a limited period.

The test is simple and can be done in the office by the examiner or his assistant with inexpensive equipment and with no loss of time during the course of a routine office examination. It is not offered as a sine qua non, but as an adjunct to be evaluated along with clinical and laboratory findings. In spite of a negative x-ray film, the test, if positive, should be carefully weighed and the patient closely watched. It may be an even more delicate index of the patient's condition than the clinical picture, leucocyte count, temperature curve, blood analysis, or x-ray film. The test is done routinely on every patient along with the general physical examination. Sometimes this cancer pattern has been the sole evidence of the presence of malignancy, and has led to a more thorough study of the patient.

Our results justify the continued use of this test as a detector of early malignant tumor formation.

REFERENCES

1. Fitz, R.: The Periodic Health Examination as a Method of Clinical Investigation, J. A. M. A. 112: 1116, 1939.
2. Piersol, G. M.: Cyclopedia of Medicine, Philadelphia, 1940, F. A. Davis Company.

3. Stahl, F. A.: Thesis: Cancer, Causes, Chicago, 1931.
4. Boyd, W.: Growth, Normal and Abnormal, J. A. M. A. **105**: 1522, 1935.
5. Fuchs, H. J.: Chemical Diagnosis of Cancer, Klin. Wchnschr. **4**: 2350, 1925.
6. Robinson, C. S., Evers, R., and Truex, A.: Fuchs Test for Malignancy, Arch. Surg. **41**: 730, 1940.
7. Rosenthal, O.: Fuchs Reaction, Am. J. Cancer **37**: 566, 1939.
8. Esculies, C., and Esculies, J.: Ether-Soluble Protein in Serum of Patients With Neoplasms, Dia Medico (Buenos Aires) **91**: 116, 1940.
9. Pfeiffer, E.: Ueber die Beeinflussung des Kristallisationsbildes des Kupferchlorids durch tuberkulöses Material, München med. Wchnschr. **85**: 92, 1938.
10. Rascher, S., and Trumpp, J.: Attempt at Crystallographic Diagnosis of Carcinoma, München med. Wchnschr. **86**: 544, 1939.
11. Gruner, O. C.: Pfeiffer's Crystallization Method for the Diagnosis of Cancer, Canad. M. A. J. **43**: 99, 1940.
12. Miley, G. P.: Crystallography, Hahnemannian Monthly, May, 1940.
13. Hirsh, J. E.: The Value of the Sedimentation Test as a Diagnostic Aid, Ann. Int. Med. **10**: 495, 1936.
14. Wolfson, S. A., Reznick, S., and Gunther, L.: Early Diagnosis of Malignant Metastases to the Spine, J. A. M. A. **116**: 1044, 1941.
15. Goldberger, E.: Rapid Bedside Test for Measuring the Blood Sedimentation Rate, New York State J. Med. **39**: 867, 1939.
16. Rubin, E. H., and Smith, N. H.: Relation of Hemoglobin, Cell Count, and Cell Volume to the Erythrocyte Sedimentation Reaction, Arch. Int. Med. **39**: 303, 1937.
17. Davison, R.: Relationship Between Plasma Proteins and Erythrocyte Sedimentation Rates in Chronic Atrophic Arthritis, J. Lab. & Clin. Med. **25**: 935, 1940.

BLOOD PRESSURE FLUCTUATIONS IN RESPIRATORY OBSTRUCTION*

Experimental Observations

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With the Technical Assistance of Robert S. Berkson, Donald W. Hall, Murl E. Kinal, and Abie S. Lenzner

IT HAS long been known that in normal persons and in animals there is a slight fluctuation of the systemic systolic blood pressure during quiet respiration.[1] In asthma patients, when the dyspnea is pronounced, it has been noted that the systolic blood pressure may fluctuate markedly during the respiratory cycle.[2-5] This fluctuation decreases as the asthmatic paroxysm subsides,[3, 4] and is usually within the normal limits of quiet respiration when the patient is asthma free.[4]

Recent literature reveals some experimental work with animals on the effects of various types of respiratory obstruction.[6-9] The reports include data on lung volume, blood changes, and the production of emphysema, but very few observations have been made of the effects of such obstruction on blood pressure.

The following experiments were carried out in order to determine whether blood pressure changes, similar to those observed in asthmatic patients, could be induced at will by obstructing respiration in animals and in man, and to detect a quantitative relationship, if any, between the amplitude of these changes and the degree of respiratory obstruction.

*From the Medical Services and Allergy Clinic of the Buffalo General Hospital.
Received for publication, March 16, 1942.

ANIMAL EXPERIMENTS

Cats were used anesthetized by chloralose rectally. Experiments were carried out on a number of animals, all of which responded similarly to the various procedures.

Simultaneous kymograph tracings were made of the carotid blood pressure, the intratracheal air pressure, the intrapleural pressure, and the respiratory movements. The blood pressure manometer was of mercury; the air pressure manometers were of water (see Fig. 1 for details of preparation of the cat). One to 3 c.c. of air were injected into the pleural cavity to prevent closure of the intrathoracic end of the blunted needle.

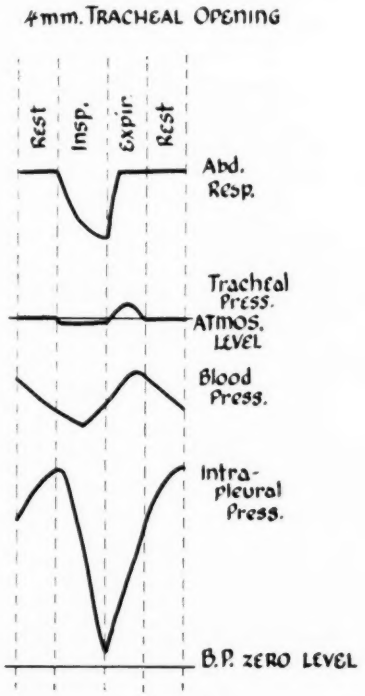

Chart 1.

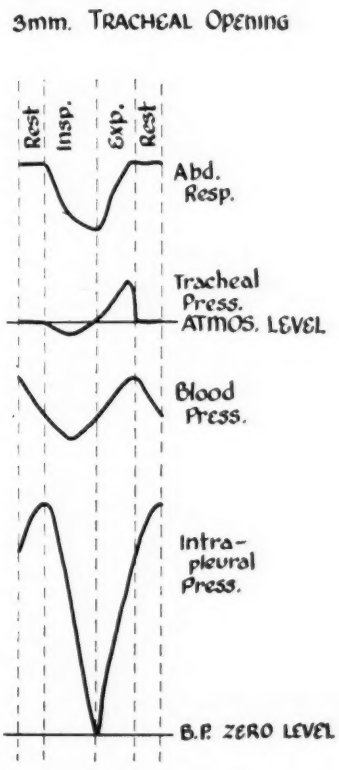

Chart 2.

Charts 1-5.—Rest, respiratory rest interval; Insp., inspiration; Exp., expiration. Scale drawings from Figures, bringing all simultaneous moments of time into the same vertical draw through the curves.

The open end of the T-shaped tracheal cannula was provided with a short rubber sleeve into which could be inserted short lengths of glass tubing with various-sized lumina. At the beginning of each experiment a glass tube with the lumen approximately equal to the diameter of the trachea was used, assuring free breathing. Successively smaller tubes were then inserted, introducing increasing obstruction to the respired air. Tracings of sufficient length were made with each size to secure reliable records. None of the cats survived with the 0.5 mm. tube in place. Tracings were continued until death.

The charts are scale drawings from the tracings, corrected so as to bring all points in any vertical line drawn through the tracings into the same moment

of time. The downstroke in the three respiratory tracings represents inspiration; the upstroke, expiration. The time line, with five-second intervals, lies at zero blood pressure level.

The following results are from continuous observations on a single cat, a healthy female, weighing 3.2 kg., anesthetized with 0.32 Gm. of chloralose.

A study of the tracings, and of the charts developed from them, will show several significant things.

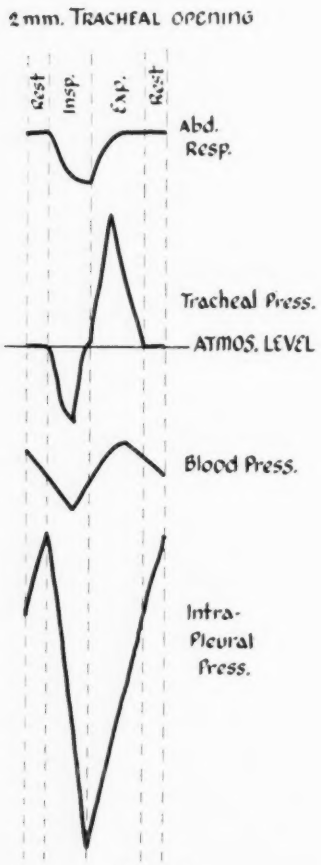

Chart 3.

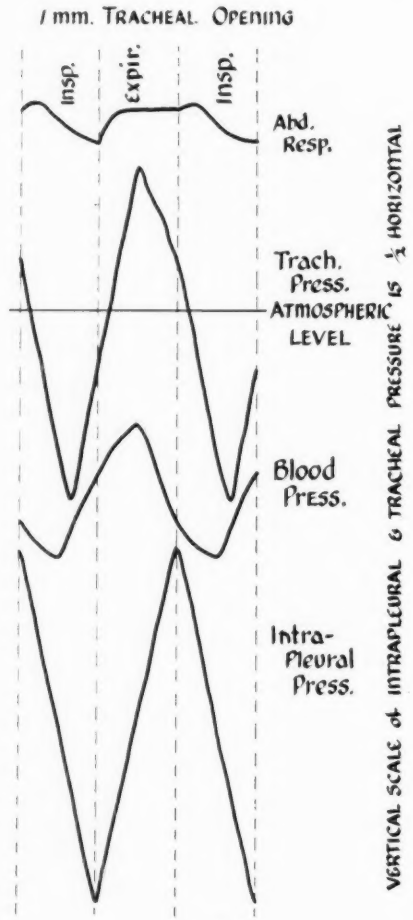

Chart 4.

With free breathing (4 mm. opening) there was a respiratory fluctuation of the blood pressure, amounting to about 4 mm. Hg. When the 3 mm. and 2 mm. openings were inserted, this fluctuation was increased slightly to about 5 mm. Hg. As would be expected, the fluctuations of the intratracheal and intrapleural pressures during respiration also increased somewhat, the increase being more noticeable in the intratracheal pressure, and with the 2 mm. opening. With the latter opening in place there was moderate respiratory obstruction.

The low point of the blood pressure, with free breathing (4 mm. opening) and with the 3 mm. and 2 mm. openings in place, occurred during inspiration, and the high point occurred during the latter part of expiration.

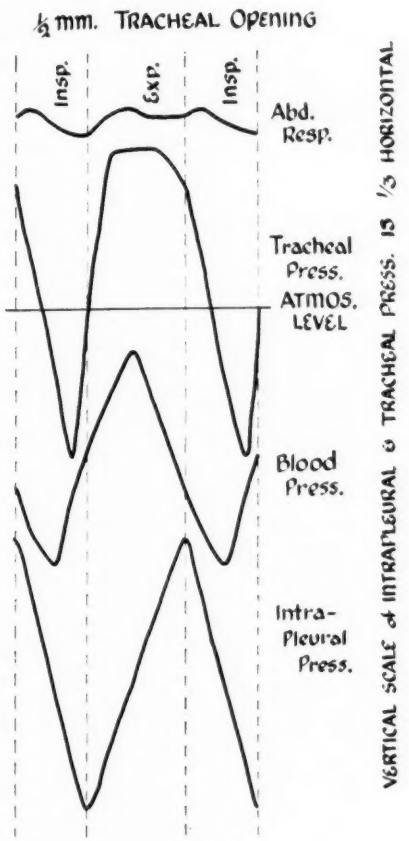

Chart 5.

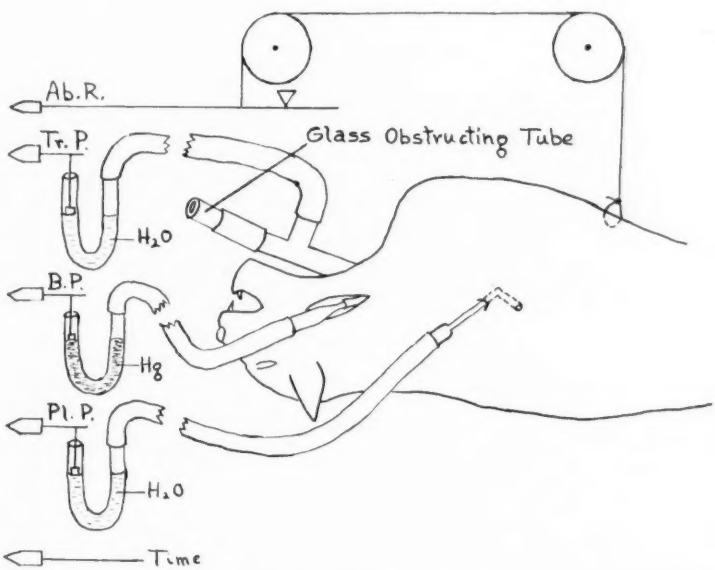

Fig. 1.—Preparation of the cat.

Figs. 1-5.—Ab. R., abdominal respiration, mechanical; Tr. P., intratracheal pressure; B.P., blood pressure, carotid; Pl. P., intrapleural pressure; Time signal indicates five-second intervals and is at blood pressure zero.

When the 1 mm. opening was inserted, inducing marked respiratory obstruction, there was an immediate increase of the blood pressure fluctuation to 10 mm. Hg. This was raised still further to 15 to 20 mm. Hg when the 0.5 mm. opening was put in place. This smallest opening presented very marked obstruction to the respired air, causing the death of the cat from asphyxia eight or ten minutes later. The low point of the blood pressure still occurred during inspiration, and the high point occurred during expiration with both of these openings, although the exact location of these points cannot be precisely determined due to the wide swings and to the inertia of the water manometers used to record the intratracheal and intrapleural pressures.

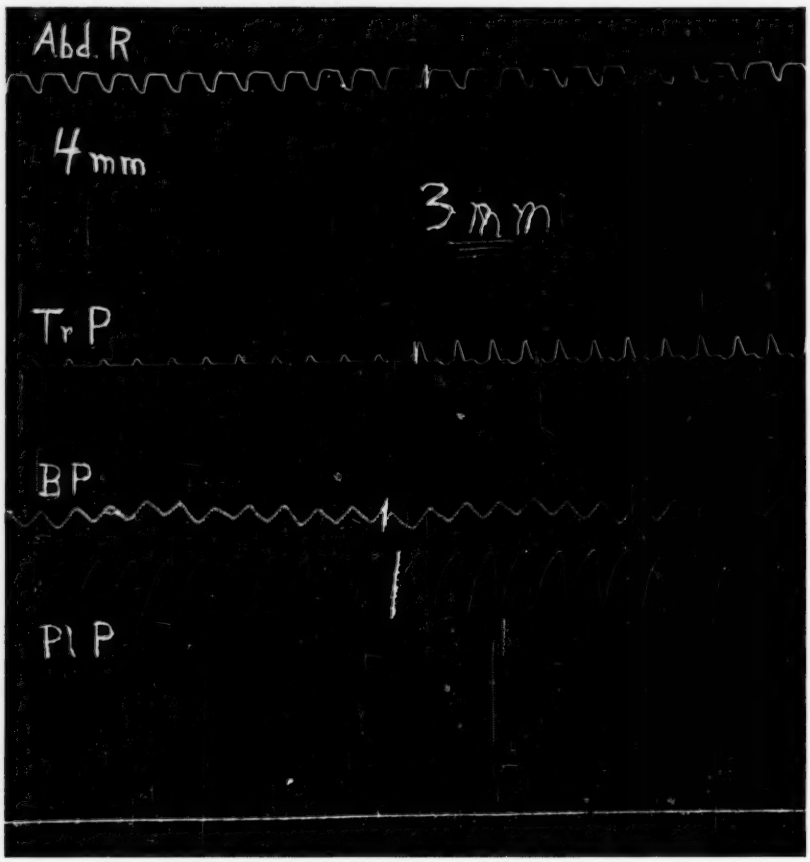

Fig. 2.

With the two smallest openings in place there was a tremendous swing of the intratracheal air pressure during inspiration and expiration, and a correspondingly large swing of the intrapleural pressure. As indicated by these two respiratory tracings, the intrathoracic pressure is greatly reduced during inspiration, and greatly increased during expiration.

SUMMARY OF FINDINGS ON CATS

The normal respiratory fluctuation of blood pressure in the anesthetized cat was increased very little when slight or moderate obstruction was presented

to the respired air at the trachea. When greater degrees of obstruction were created, the amplitude of this fluctuation increased two to four times; i.e., beyond a certain point, the greater the obstruction, the greater the blood pressure fluctuation.

The maximum and minimum blood pressure fluctuations occurred in the same phases of the respiratory cycle, whatever the degree of respiratory obstruction; i.e., the low point occurred during inspiration, and the high point during expiration.

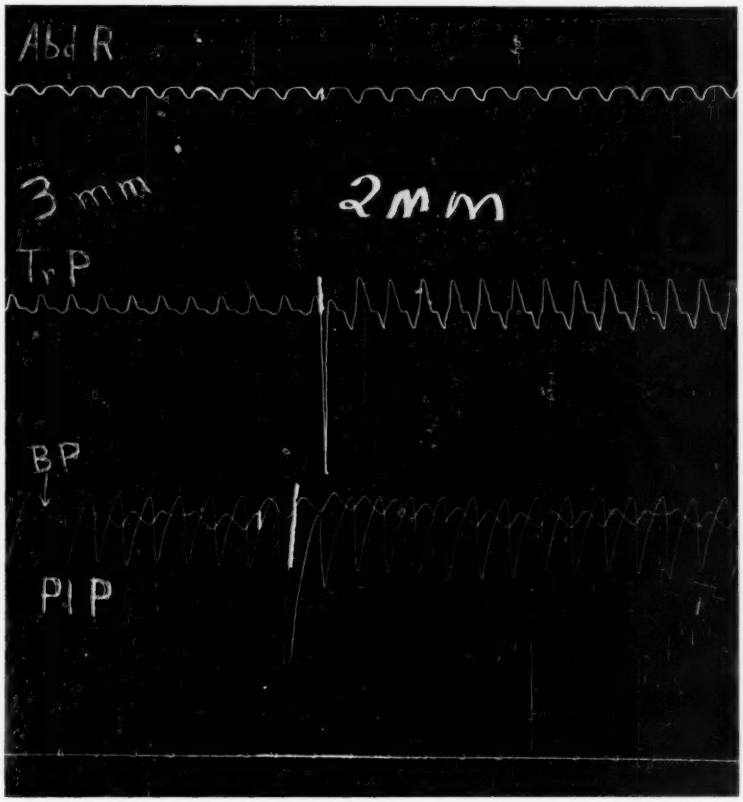

Fig. 3.

HUMAN EXPERIMENTS

In human beings fluctuations of the systolic blood pressure, synchronous with respiration, may be observed with considerable accuracy, using an ordinary sphygmomanometer. The 13 cm. cuff about the upper arm is inflated to above the systolic pressure. It is then slowly deflated (not over 2 or 3 mm. Hg per breath) while the respiratory movements are watched. At the high point of the systolic respiratory fluctuation a few arterial beats will be heard in the antecubital space by auscultation, followed by a trough of silence. If the cuff pressure is further lowered slowly, beats will come through more and more until they are heard throughout inspiration and expiration. The pressure reading where they are just heard throughout both phases of respiration marks

the low point of the fluctuation. On quiet breathing in normal persons, the fluctuation of the systolic blood pressure amounts to between 2 mm. and 6 mm. Hg, with the high point during expiration, and the low point during inspiration.

TABLE I

RESULTS OF BREATHING THROUGH CONSTRICTED MOUTHPIECE

APERTURE	TIME	SYSTOLIC B.P.		FLUCTUATION	DIASTOLIC
		EXPIRATION	INSPIRATION		
Subject William F.					
Full	11:35	122	120	2	74
$\frac{1}{4}'' \times \frac{3}{4}''$	11:37	114	112	2	80
$\frac{3}{8}'' \times \frac{5}{8}''$	11:39	116	112	4	78
$\frac{1}{8}'' \times \frac{1}{2}''$	11:42	114	114	0	78
$\frac{1}{8}'' \times \frac{1}{16}''$	11:44	130	102	28	
		138	98	40	
		150	120	30	
		150	120	30	
No cyanosis noticeable					
Subject Walter F.					
No mouthpiece	12:14	134	130	4	76
$\frac{3}{4}'' \times \frac{3}{4}''$	12:16	120	116	4	80
$\frac{1}{8}'' \times \frac{3}{16}''$	12:18	134	120	14	
		140	122	18	
$\frac{1}{8}'' \times \frac{1}{16}''$	12:20	150	114	36	
Very slight cyanosis with smallest aperture					
Subject A. F. L.					
No mouthpiece		115	112	3	76
$\frac{1}{16}'' \times \frac{1}{4}''$		135	122	13	78
No cyanosis					

TABLE II

RESULTS OF HYPERVENTILATION WITH FREE BREATHING

	SYSTOLIC B.P.		FLUCTUATION	DIASTOLIC
	EXPIRATION	INSPIRATION		
Subject A. F. L.				
Quiet respiration	115	112	3	76
	124	122	2	80
Hyperventilation	124	122	2	
	120	117	3	
Subject A. R. R.				
Quiet respiration	123	120	3	74
Hyperventilation	108	104	4	
	104	98	6	
Subject W. F.				
Quiet respiration	130	126	4	86
Hyperventilation	140	134	6	
	134	130	4	
Rest (quiet breathing)				
Hyperventilation	126	120	6	
Hyperventilation continued	118	104	14	
for one to two minutes	118	106	12	Slight dizziness

Healthy young medical students in their twenties were used for the following experiments, using the foregoing method for determining the fluctuation of the systolic blood pressure. The subjects were recumbent.

With the nose closed with a clip and a wide rubber mouthpiece, bearing a thumb-screw pinchcock, held tightly in the mouth, the respiratory fluctuations of the systolic blood pressure were determined, first on free breathing, then with the mouthpiece narrowed in successive stages.

With the smaller apertures there was marked obstruction to respiration, and the respiratory rate was slowed to permit the passage of the tidal air. In all three subjects the low point of the blood pressure fluctuation came during inspiration, and the high point during expiration, both in free breathing and with all degrees of obstruction.

Since the respiratory efforts in breathing through small apertures resembled those of hyperventilation, blood pressure readings were made on healthy subjects with unobstructed breathing, both during quiet respiration and during voluntary hyperventilation.

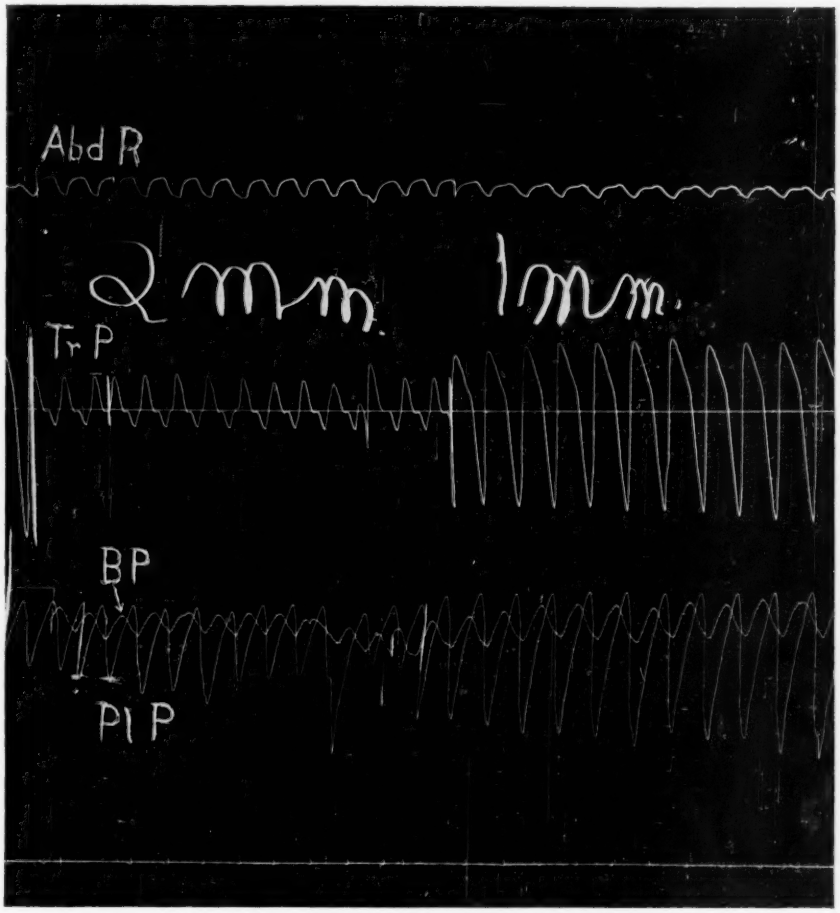

Fig. 4

As with the experiments with obstruction, likewise in hyperventilation, the low point of the systolic blood pressure fluctuation occurred during inspiration, and the high point during expiration.

Obstruction to respiration at the mouth caused an increase in the respiratory fluctuation of the systolic blood pressure roughly parallel to the degree of obstruction, as in the cat experiments. Hyperventilation alone, on the other hand, caused no increase in this blood pressure fluctuation in two subjects. In the third there was a moderate increase after hyperventilation had continued from one to two minutes.

DISCUSSION

Prinzmetal[8] showed that the normal, slightly negative intrapleural pressure became more negative in asthmatic patients during the paroxysm, although he did not describe the fluctuations of this pressure during each respiratory cycle. In our cat experiments the intrapleural pressure fluctuated to an increasingly greater negativity during inspiration and showed an increasing, though less marked, movement toward the positive side during expiration, as the tracheal obstruction was increased.

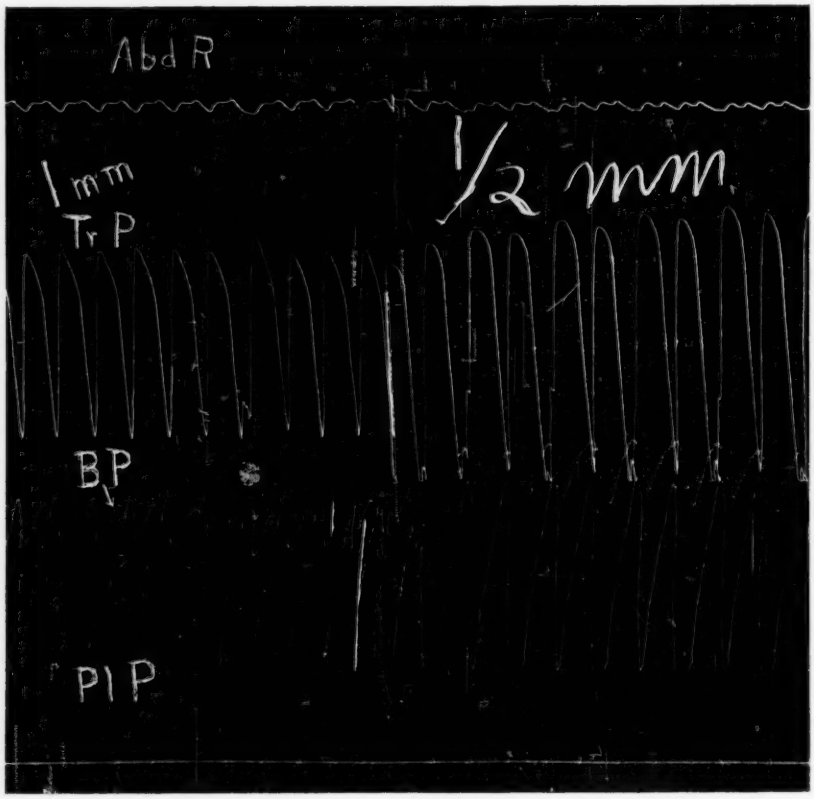

Fig. 5.

It would seem, therefore, that the fluctuations of the systolic blood pressure under respiratory obstruction, observed in our experiments, are closely related to the concurrent changes of the intrathoracic pressure during the respiratory cycle, and that their amplitude reflects roughly the degree of respiratory obstruction.

It would be unsafe to apply directly the results of these animal and human experiments to an explanation of the respiratory fluctuations of the systolic blood pressure observed in asthmatic patients, since the site of the obstruction to respiration differs in the two cases. Nevertheless, there is obstruction to breathing in bronchial asthma; and it has been shown[8] that the intrathoracic pressure deviates from the normal in this condition. Moreover, unpublished

observations which I have made show that the blood pressure fluctuations in asthma closely resemble those seen in our present experiments, both in their timing during the respiratory cycle, and in the parallelism between their amplitude and the degree of respiratory embarrassment. The assumption to be drawn, therefore, is that forces are in action in bronchial asthma, similar to those in the experiments here described and that these forces have a causal relationship to the observed blood pressure fluctuations.

CONCLUSIONS

Cat Experiments

1. The greater the obstruction at the trachea to passage of the respired air, the greater the fluctuation of the blood pressure during the respiratory cycle.

2. Whatever the degree of respiratory obstruction, the high point of the blood pressure occurs during expiration, and the low point during inspiration.

Human Experiments

1. Marked respiratory obstruction at the mouth induces greatly increased respiratory fluctuation of the systolic blood pressure.

2. With this obstruction in place the high point of systolic blood pressure occurs during expiration, and the low point during inspiration.

I wish to express my appreciation to Dr. Frederick Reece Griffith, Jr., Professor of Physiology, University of Buffalo School of Medicine, for much encouragement and advice, and for his hospitality in making available for this work the facilities and resources of the laboratories of his Department.

REFERENCES

1. Wiggers, C. J.: Physiology in Health and Disease, Philadelphia, 1934, Lea & Febiger, page 532.
2. Sihle, M.: Zur pathologie und therapie des asthma, Wien. klin. Wchnschr. **16**: 85, 1903.
3. Rackemann, F. M.: Clinical Allergy, New York, 1931, The Macmillan Co., page 431.
4. Tinel, J., and Jacquelin, A.: Les modifications circulatoires peripherique au cours de l'acces d'asthme, Bull. et mém. Soc. méd. d. hôp. de Paris **47**: 440, 1931.
5. Feinberg, S. M.: Allergy in General Practice, Philadelphia, 1934, Lea & Febiger, page 99.
6. Hoover, C. F. : Tracheal and Bronchial Stenosis as Causes for Emphysema, Arch. Int. Med. **29**: 143, 1922.
7. Moore, R. L., and Binger, C. A. L.: Response to Respiratory Resistance; Comparison of Effects Produced by Partial Obstruction in Inspiratory and Expiratory Phases of Respiration, J. Exper. Med. **45**: 1065, 1927.
8. Prinzmetal, M.: Relation of Inspiratory Distention of the Lungs to Emphysema, J. Allergy **5**: 493, 1934.
9. Blalock, A., Harrison, T. R., and Wilson, C. P.: Partial Tracheal Obstruction; An Experimental Study in the Effects on the Circulation and Respiration of Morphinized Dogs, Arch. Surg. **13**: 81, 1926.
10. Wiggers, C. J.: loc. cit., page 537.

THE TREATMENT OF SEVERE PERIODIC HEADACHES WITH "DESENSITIZING" DOSES OF PROSTIGMINE*

A New Conception and Treatment of Certain Types of Headaches Including Those Designated as Migraine and Histamine Headaches

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OF THE many ills to which humanity is heir, headache is one of the most common. Even after arbitrarily dismissing those types due to increased intracranial pressure (brain lesions and hypertension), sinusitis, anemia, and the so-called "nervous" headaches, there still exists an extremely large group of periodic headaches loosely termed "migraine."

Brickner[1] has described "migraine headache" as a severe periodically recurring, unilateral headache which lasts from a few hours to several days, and is often accompanied by nausea and vomiting. Not infrequently the attack is preceded by an aura, consisting of some form of visual disturbance, and in many instances a familial history of migraine or allergy is present. In women the attacks frequently occur at the menstrual periods.

A considerable number of these "migraine" headaches have recently been reclassified by Horton and others[2] as histamine cephalalgias because they can be reproduced by histamine injections and can be controlled by desensitizing the patient with small but increasing doses of histamine. Typically, this new class of headache is characterized by a very severe unilateral pain of a burning, boring nature, which may involve the same side of the face. The conjunctiva on this side is often injected and the face is usually flushed. In general, the attacks occur more frequently, but are of shorter duration than the migraine type—lasting from fifteen minutes to several hours. Further points of difference exist in that they are not preceded by auras, vomiting is rare, and they are usually confined to the older age group.

The present work has been motivated by the desire (1) to extend the group of vascular headaches described by Horton to all those manifesting a marked skin reaction to histamine or acetylcholine; (2) to demonstrate a new method of producing these headaches; and (3) to present a new, very simple and extremely encouraging method of treating them.

The original stimulus to the present study was supplied by a chance observation in a patient with a typical histamine headache, as described by Horton. Several weeks subsequent to the cessation of histamine desensitization therapy,

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Received for publication, February 2, 1942.

this patient suffered an attack which was aborted with remarkable promptness by the administration of one tablet of prostigmine bromide. Some time later, when his desensitization had apparently lapsed and he was on the verge of another headache, similar treatment with prostigmine produced a typical, very severe, cephalalgia, accompanied by fullness in the back of the head, diarrhea, abdominal cramps, and a mild attack of wheezing. It would appear, therefore, that prostigmine was able to duplicate in this patient with a typical "histamine cephalalgia," the actions of histamine in both the desensitized and sensitive phases. This observation was deemed of particular significance in view of the fact that Horton and others,[2] in summarizing his excellent work, stated, "The spectacular manner in which patients respond indicates that histamine treatment is as specific for this syndrome as insulin is in the treatment of diabetes mellitus."

Another hint that histamine might not be the only factor involved in this type of headache was demonstrated by another patient who failed to respond—even after two hours—to epinephrine (0.5 c.c. of 1:1,000 solution), whereas 1 c.c. of bellafolin (atropine-like in action) promptly alleviated the attack. To further confuse the issue, a preparation of histaminase (torantil), which effectively served to decrease the incidence of attacks in this patient one year ago, proved to be valueless in recent months.

In the light of these observations it was decided to investigate the effect of prostigmine on headaches of the migraine and histamine types with a view toward expanding the present knowledge of the causal relationships and the etiologic factors underlying these headaches. Accordingly, a series of 60 cases of headaches were carefully analyzed, and for the purpose of convenience were divided, symptomatically, into four principal groups. Some variations existed within each group since, obviously, though the same neurobiochemical mechanism might conceivably be operative in every case, the symptomatology was dependent upon such variables as the psychic, somatic, and endocrinologic factors. The groups were divided as follows:

Group I consisted of 20 patients with more or less typical histamine cephalalgias, who frequently manifested, in addition, such symptoms as fullness in the head, ears, and back of the neck, pain in the muscles of the neck, and dizziness.

Group II consisted of 25 patients with the "migraine" type. The headaches in this group were of longer duration than those in group I and were frequently preceded by auras and accompanied by vomiting. The patients were of the younger age group with headaches since childhood and frequently with familial histories of allergy, migraine, or premature graying of the hair.

Group III consisted of 8 patients who complained of a constant tightness and fullness in the back of the neck which did not alternate with the headaches described by Horton. Dizziness was a prominent feature in these cases, and occasionally there were full-blown attacks of Ménière's syndrome. In 2 of these patients the headaches were previously ascribed to hypertension, although the systolic pressure was 150 to 160 mm.

Group IV consisted of 7 patients with hypertension of whom 3 had systolic blood pressure of 200 mm. or more, with pounding of the vertex of the skull and fullness or tightness in the neck muscles. The remaining 4 were in the

hypotensive phase without cardiac decompensation. In these, the fullness in the head and ears, and the tightness of the neck muscles were very marked.

The common denominator in all the above groups was a marked cutaneous sensitivity to histamine and/or acetylcholine. All patients reacted markedly to 0.01 mg. of histamine (0.01 c.c. of a 1:1,000 solution) injected intradermally, by exhibiting a large wheal, pseudopodia, and an erythematous flare of 2 or more inches.* Many also gave a marked reaction to 1 mg. of acetylcholine (0.02 c.c. of a 1:20 solution). Indeed, in some instances, sensitivity to acetylcholine was more pronounced than to histamine. Many patients were also allergic to some antigen.

TREATMENT

The notable sensitivity to acetylcholine was regarded as presumptive evidence of the possibility that acetylcholine might be a factor in the production of these headaches. It was hoped, therefore, that these patients could be "desensitized"—through a mechanism parallel to that operative in histamine "desensitization"—by the oral administration of increasing doses of prostigmine bromide, a parasympatheticomimetic drug, which is known to mobilize the acetylcholine reserves of the body by inhibiting the action of the acetylcholine esterase. Accordingly, a 15 mg. tablet of prostigmine bromide was dissolved in one ounce of water, and the resulting solution was administered as follows: On the first day, 1, 2, and 3 drops were given at morning, noon, and night, respectively. The increase of 1 drop per dose was maintained until 30 drops was reached, following which 30 drops were given each day for one week and then three times per week for an indefinite period. Occasionally, when it was felt that the results could be improved, the dose was increased to 40 drops. A few of the patients were given these drops in a slower fashion, e.g., the same dosage was given morning and night, and was increased by one drop each day until the dose of 30 drops was reached. Then the usual procedure was followed. This method was used when it was felt—on the basis of skin tests—that the patient was unduly sensitive to histamine or acetylcholine. For the purpose of uniformity in this experiment the majority of the patients were given identical dropper bottles containing previously prepared solutions.

The patients were initially skin tested with histamine before the institution of treatment, and repeat tests were made at approximately weekly or fortnightly intervals. Some were also tested with acetylcholine. Care was taken to swab the skin lightly with alcohol before the intradermal injection because vigorous rubbing in itself could produce a reaction in sensitive persons. A 26 gauge Schick needle with a tuberculin syringe was used for the test. The test was read arbitrarily five minutes after injection, traced on cellophane with a skin pencil, and retained for future records.

RESULTS

Significantly, in 40 per cent of the cases the skin reaction to injected histamine decreased, i.e., the size of the wheal was reduced, and the flare dimin-

*Since nearly all persons give some reaction to intracutaneously injected histamine, it has been necessary to employ this criterion for a marked reaction. (Atkinson, M.: Observations on Etiology and Treatment of Ménière's Syndrome, J.A.M.A. **116**: 1753, 1941.)

ished or disappeared by the treatment with "desensitizing" doses of prostigmine. Often in the initial histamine skin test there was marked localized itching, a symptom which was not evident in subsequent skin tests after the patients developed tolerance. There was no correlation, however, between the size of the wheal at a given time and the measure of relief. Of especial significance was the fact that the acetylcholine wheal and flare were always reduced in size following treatment. After one week the time taken for disappearance of the acetylcholine flare from the skin was usually halved.

TABLE I

GROUP	TYPE	LITTLE OR NO RELIEF	MODERATE RELIEF	MARKED TO COMPLETE RELIEF	TOTAL NUMBER OF CASES
I	"Histamine" headache	2	1	17	20
II	"Other migraine types" of headache	2	1	22*	25
III	Fullness in back of neck and dizziness + occasional attacks of Ménière's syndrome	0	0	8	8
IV	Hypertension with fullness in back of neck. Experimental	0	3	4	7

*Since the acceptance of this paper, 11 (50 per cent) of these patients have reverted to the "moderate relief" designation. The reason for this is not clear but is being investigated.

All the patients exhibiting definite periodic headaches, including those similar to the headache described by Horton, and those usually classed as migraine were relieved in large measure. The relief was definite, dramatic, and in many cases complete, for the duration of time so far observed. The results are recorded in Table I. The figures are based on patients' estimates of relief of their headaches, and are, therefore, rather arbitrary. Several persons independently observed that even though a mild headache did occur, it could be dispelled in a few minutes by taking 15 drops of the solution.

In the patients with hypertension, that part of the headache described as fullness in the head, with the complaint that the head was twice the normal size, and the feeling of tightness of the neck muscles were relieved in several cases. The part ascribable to the hypertension, i.e., the pounding of the vertex of the skull, was not improved. The blood pressure was not reduced by this medication, but some patients with hypertension had only the fullness of the head and felt well once this was gone. The patient with hypertension in the hypotensive phase was usually completely relieved of symptoms, although the blood pressure did not rise.

CASE REPORTS

CASE 1.—C. M., male, aged 31 years, had a history of three years of severe, unilateral, burning and boring type of headaches, which lasted from several hours to a full day. These occurred about twice a week. There was no vomiting or aura. The patient's eyes teared, and his face appeared flushed on the side of the headache. For two months prior to treatment the headache appeared about four or more times a week and completely disabled him. The patient noticed that alcohol, even in slight quantities, could bring out a severe headache.

During the past year he has had three attacks of fullness in the back of the neck, coupled with dizziness that lasted all day. His previous history revealed a healed tuberculosis. His father has osseous tuberculosis. On May 25, 1941, the patient was put on prostigmine in desensitizing doses. On June 1 he had a slight headache over the whole forehead, but no unilateral headache. On June 8 he had one mild headache over the whole forehead, but again he had no unilateral headache. On June 15 he had one mild unilateral headache. The patient was last heard from on Oct. 1, 1941, at which time he stated that he had had no more unilateral headaches. Occasionally, after taking a drink of liquor, he developed a sensation as if a headache were about to come, but it never materialized. He was told to continue the medication. The histamine wheals are shown in Fig. 1.

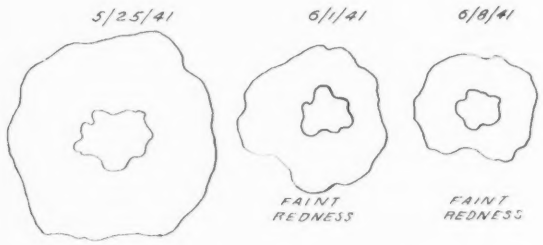


Fig. 1.—Histamine wheals (one-fourth actual size). Case 1, C. M.

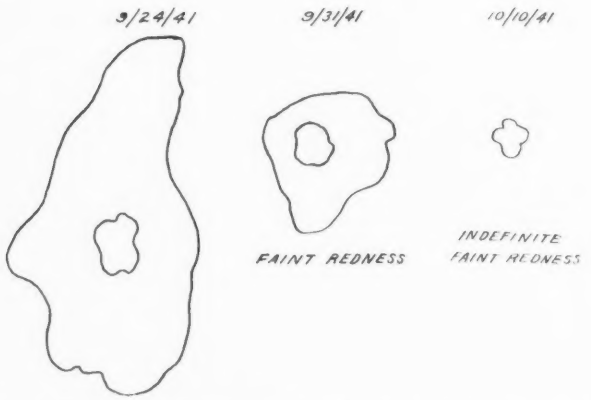


Fig. 2.—Acetylcholine wheals (one-fourth actual size). Case 2, L. P.

CASE 2.—L. P., female, aged 30 years, gave a history of severe unilateral headaches since childhood. The headaches usually lasted for about eight hours and culminated in vomiting. There was no lacrimation or flushing of the face. Occasionally there were some preheadache phenomena of slight blurring of vision. The only time in recent years that the patient has been free of headaches was during her two pregnancies, and a short interval when histaminase appeared to diminish the frequency of attacks. These severe headaches had been occurring at the rate of three to four times a week during the month previous to institution of treatment. They were especially frequent during shopping tours. The patient was also extremely reactive to soap, large blotches appearing on her skin after using any soap. The family history revealed that her brother has severe hay fever, and her sister has many attacks of vomiting and unilateral headaches that are not severe and last a short time. Her physical examination revealed a blood pressure of 120/90, normal eye grounds, normal refraction of the eyes, and normal ear, nose, and throat findings. On Sept. 24, 1941, following recovery from a very severe headache, she was skin tested with acetylcholine intradermally and prostigmine bromide was started in "desensitizing" doses. On September 31 she reported that

she had had no headache for a week, though at one time during this week she experienced some slight blurring of vision which did not, however, result in the usual severe headache. On October 5 she experienced a slight frontal headache, which did not last long and yielded readily to one aspirin tablet. When she was skin tested on October 10, she stated that she had had no severe unilateral headaches since the treatment. She also volunteered that she had ceased to be sensitive to soap. She was last seen on Dec. 1, 1941, and at that time she stated that she had not had the severe, unilateral type of headache that she had experienced before treatment. However, on two occasions she experienced an extremely mild unilateral fullness of the head but it did not incapacitate her. She was urged to continue the treatment. The acetylcholine wheals are shown in Fig. 2.

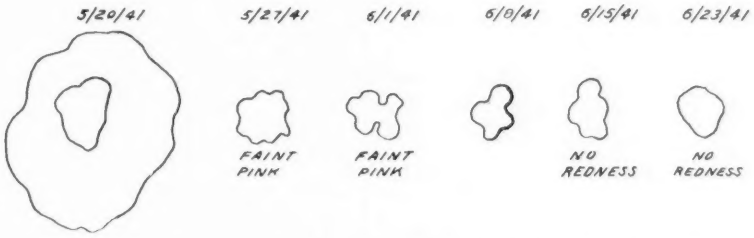


Fig. 3.—Histamine wheals (one-fourth actual size). Case 3, E. H.

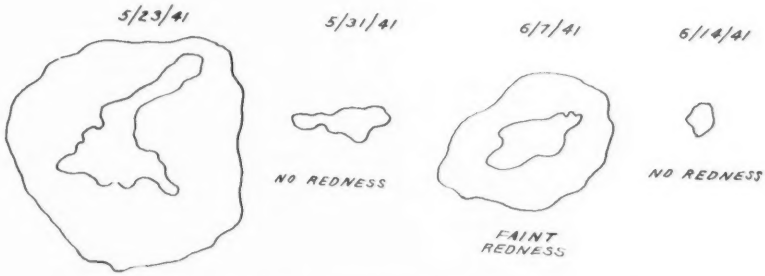


Fig. 4.—Histamine wheals (one-fourth actual size). Case 4, F. C.

Case 3.—E. H., male, aged 27 years, stated that for three years he had frequent attacks of fullness of the head, dizziness, and tinnitus. In addition, practically every day he had the feeling of a "clouded" head, that reached a maximum in the afternoon. He was previously told that his blood pressure was 160, and that his headache was due to this. No treatment seemed to be of any avail. He presented an endocrine problem because of a voluminous supply of hair all over his body, coupled with underdeveloped genital organs. The physical examination revealed a blood pressure of 140/90, the eye grounds were normal, and the refraction was normal. On May 25, 1941, prostigmine was started in "desensitizing" doses. On June 1 the patient reported that he had had no fullness of the head at any time during the preceding week. On June 8 he stated that slight fullness was present on two days during the past week. He was told to increase the dosage to 40 drops and then take 40 drops a day. The blood pressure was 142/90. On June 15 the patient reported no fullness of the head or dizziness and stated that he felt very well. The blood pressure was 134/90. The patient was last seen on Sept. 15, 1941, at which time he did not have any fullness or clouding of the head. Several times during the past month he had a slight ringing in one of his ears; this vanished within a few moments. He has been told to continue taking the medication. The histamine wheals are shown in Fig. 3.

Case 4.—F. C., female, aged 36 years, had attacks of fullness in the back of her neck, dizziness, and ringing in both ears for many years. These attacks recurred about two times a week for a few years. Her anamnesis had a marked hysterical taint; she was in constant

conflict with her husband because she desired to become pregnant and he refused to consider it. Previous history revealed that she was on a Bradford frame for many months in an orthopedic hospital, but the x-ray examination and physical signs revealed no osseous tuberculosis. On May 23, 1941, the physical examination revealed a blood pressure of 120/80, normal eye grounds, normal refraction, and normal nose and throat findings. On the same day prostigmine was started in "desensitizing" doses. On May 31 there was neither fullness nor dizziness. On June 7 the patient was well. By August 5 the prostigmine had been stopped one and one-half months without return of the symptoms in her head. On October 5, when she had not taken medication for approximately three months, there was slight return of the fullness of the head and dizziness. She was put on the "desensitizing" dosage of prostigmine. On October 12, 1941, when she was last seen, there was no return of the fullness since taking medication. The histamine wheals are shown in Fig. 4.

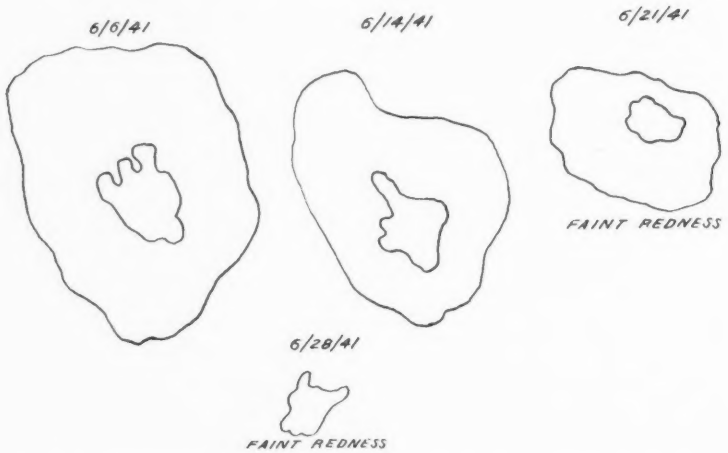


Fig. 5.—Histamine wheals (one-fourth actual size). Case 5, F. B.

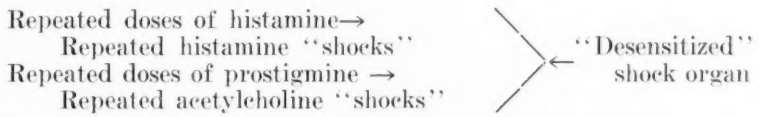
CASE 5.—F. B., female, aged 41 years, came to the Jewish Hospital Clinic on June 6, 1941, with a history of constant fullness in her head, especially in the back of it, for the past four weeks. She also had occasional fullness in her ears. The head felt as if it were twice the normal size. The previous history revealed that on April 30, 1936, the blood pressure was 204/130, and that the cardiac outline was enlarged. On March 22, 1941, the blood pressure was 175/125, and on May 31, 1941, it was 150/100. Her menstrual periods were irregular. The physical examination revealed that she had a blood pressure of 145/100 at this visit and that there were normal eye grounds and normal refraction. On May 31 prostigmine was started in "desensitizing" doses. On June 14 she reported no fullness in the head. The blood pressure was 140/100. On June 21 she reported that there still was no fullness in the head. On June 28 she reported occasional frontal headaches but no fullness or "large head." On Oct. 1, 1941, when she was last seen, she reported that she felt perfectly well and had none of her original symptoms. Her blood pressure at that time was 150/100. The histamine wheals are shown in Fig. 5.

COMMENT

The results obtained in this study serve to extrapolate the well-known parallelism between histamine and acetylcholine[3] to the field of periodic headaches. Both of these substances are native to the body and both of them have been accused by different investigators of being the "allergic" substance. Histamine is produced by toxic or mechanical injuries of tissue cells, and acetyl-

choline results from the stimulation of the cell by the nerves. Acetylcholine would, therefore, appear to be the substance usually present unless some form of injury is presupposed. In addition, it would now appear that both histamine and acetylcholine may be causal factors in periodic headaches either at the same time or at different times. Certainly, both histamine and prostigmine are effective in the prophylactic treatment of these headaches.

These phenomena are of sufficient import to warrant the application of considerable thought to the question of the theoretical bases for the action of prostigmine. Admittedly, the data available are inadequate to permit the formulation, with any degree of certainty, of an all-embracing, coherent theory. Nevertheless, several explanations appear plausible enough to merit attention. The first of them is based on the known ability of prostigmine to liberate and to prolong the action of acetylcholine in the body by inhibiting the acetylcholine esterase. Thus the minute, controlled and ever-increasing quantities of acetylcholine liberated by the "desensitizing" doses of prostigmine may serve to administer mild repeated "shocks" to the hypersensitive organ responsible for the headaches, with the result that a relative tolerance or insensitivity is developed in this so-called "shock organ." This mechanism is sensibly analogous to that believed by many to be operative in histamine "desensitization." The analogy is perhaps best represented as follows:



Another explanation is based on the assumption that a person in the "sensitive" phase is in a state of autonomic or vasomotor imbalance with an ascendant parasympathetic system. This increased activity of the parasympathetic division accounts for the general hypersensitivity of the person toward both histamine and acetylcholine. Under such circumstances it may be postulated that prostigmine—though a para sympathetic stimulant in physiologic doses—may, in the minute desensitizing doses, increase the resistance of the parasympathetic division to both substances.

SUMMARY AND CONCLUSIONS

A series of 60 patients with periodic headaches with migraines and histamine cephalalgias predominating, were treated orally with gradually increasing doses of prostigmine. The extremely gratifying results which were obtained tend to prove (1) that in addition to histamine, acetylcholine is one of the factors involved in the production of these headaches; (2) that a similar neurobiochemical mechanism is operative in many types of periodic headaches; (3) that prostigmine is as effective as histamine, and more convenient than it, in the treatment of these headaches.

We wish to express our appreciation to Hoffman-La Roche, Inc., for the materials used in this study.

REFERENCES

1. Brickner, R. M.: Migraine and Other Headaches.
   Tice, F.: Practice of Medicine, Hagerstown, Md., 1937, W. F. Prior Co., Vol. 10, Chapter 12, p. 281.
2. Horton, B. T., MacLean, A. R., and Craig, W. McK.: A New Syndrome of Vascular Headache: Results of Treatment With Histamine: Preliminary Report, Proc. Staff Meet., Mayo Clin. **14**: 257, 1939.
   Horton, B. T.: The Use of Histamine in the Treatment of Specific Types of Headaches, J. A. M. A. **116**: 377, 1941.
3. Rackemann, F. M.: Allergic Diseases With Specific Reference to Histamine and Acetylcholine: Report on Medical Progress, New England J. Med. **222**: 674, 1940.

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OBSERVATIONS ON THE EFFECT OF HISTAMINE PHOSPHATE ON CAPILLARY PERMEABILITY AND INFLAMMATION*

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THE reactions of the skin to histamine are adequately described, and the observations of others are reviewed by Lewis.[1] When introduced into the skin in minute quantities, this poison "produces a characteristic series of independent phenomena comprising (a) local dilatation of the minute blood vessels by a direct action upon them; (b) a widespread dilatation of neighboring strong arterioles through the medium of a local reflex mechanism; and (c) increased permeability of the vessel walls by direct action."[1] Most investigators agree with these observations. In addition to these changes, Moon[2] has emphasized that there is stasis of blood with some diapedesis of red blood cells into the tissue spaces. The effects of histamine upon the systemic circulation are the same, and are due to the same mechanism as its local effects upon the capillaries of the skin.[2]

Bloom[3] in 1922 advanced the idea that the migration of leucocytes into the tissues in areas of inflammation may be due to histamine. Grant and Wood[4] were unable to demonstrate such an effect on the leucocytes of man, rabbit, and the frog. On the contrary, however, Wolf[5] and Findlay[6] showed that histamine acted as a positive chemotactic substance both in vitro and in vivo.

Best and McHenry[7] in 1931, in reviewing the role of histamine in inflammation, state that "the very interesting and fundamental question of the role of histamine or a histamine-like substance in the production of the phenomena observed in inflammation is still controversial. A thorough investigation of the subject is needed."

Interest in the role of histamine in the development of the local inflammatory reaction has been stimulated by the recent work of Menkin.[8] This investigator concludes from his experimental work that leucotaxine is the sub-

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Aided by grants from the International Cancer Research Foundation and the University of Tennessee.
Received for publication, March 30, 1942.

stance responsible for the increase in capillary permeability and also for the localization of leucocytes in the local area. Menkin[9] says "in regard to the statement that various products of protein metabolism, such as amino acids, are capable of inducing leucocytic migration, it is interesting to note that observations with a number of representative amino acids (as well as histamine) have failed to reveal leucocytic migration." Furthermore, there is no evidence according to Menkin to support the contention that the permeability factor in inflammation is histamine.[9]

It has been shown that trypan blue following an intravenous injection localizes and concentrates in areas of inflammation in the skin of the rabbit only during a specific interval following the application of xylol to the skin. The greatest quantity of dye localizes and concentrates in the area of skin where the xylol is applied for the shortest time before the dye is injected. It has also been shown that the localization of leucocytes in areas of skin in the rabbit, showing macroscopic evidences of inflammation, is not determined by the time that trypan blue localizes and concentrates in the same tissue.[10, 11]

A problem is outlined to study the effect of histamine phosphate on capillary permeability and inflammation because there is some question as to the role that histamine may play in these processes. The technique is similar to that used in other studies on capillary permeability and inflammation.[12-15] Varying dilutions of histamine phosphate are injected intradermally preceding the intravenous injection of trypan blue. Observations are made on the localization and the concentration of dye in the histamine-injected areas of skin. Histologic studies are also made on the areas of skin injected with the histamine. Data are given on the changes produced in the number of circulating leucocytes following an intraveous injection of histamine.

METHODS AND MATERIAL

Adult rabbits are used. The hair is carefully shaven from the sides and the abdomen several days before the experiment is begun. The following dilutions of histamine phosphate* are used: 1:1,000 (stock solution), 1:3,000, 1:5,000, 1:8,000, and 1:10,000. All dilutions are made in saline. Two-tenths cubic centimeter is injected intradermally. Several injections are made into different areas of the same animal. In some rabbits a single dilution is used while in others several dilutions are injected. Ten cubic centimeters of a 0.2 per cent solution of trypan blue in saline is injected intravenously following the last injection of the histamine. The time at which the histamine and the dye are injected is given in the individual experiments. Observations are made on the time of the localization and the concentration of the dye in the different areas of skin.

Areas of skin injected with the histamine are fixed in a 10 per cent solution of formalin immediately after the rabbits are killed. Paraffin sections are prepared and stained with hematoxylin and eosin.

Eight rabbits are used to study the effect of histamine on the number of circulating leucocytes. A standard technique is used for making these counts.

*Supplied by Parke, Davis & Company.

The white blood cells are counted in each rabbit immediately before 0.4 c.c. of a 1:1,000 solution of histamine phosphate is injected intravenously into the marginal ear vein. The white blood cells are counted after one, two, four, five, seven, twenty-three, and twenty-eight hours. These data are analyzed statistically.

TABLE I

INTERVAL BETWEEN THE INTRADERMAL INJECTION OF HISTAMINE AND THE INTRAVENOUS INJECTION OF TRYPAN BLUE

NUMBER OF RABBIT	MINUTES				HOURS				
	0	15	30	60	2	3	4	5	6
1:10,000 Dilution of Histamine									
967	0	0	0	0	0	0	0	0	0
968	+	+	0	0	0	0	0	0	0
969	+	0	0	0	0	-	-	-	-
976	+	+	+	-	-	-	-	-	-
1:8,000 Dilution of Histamine									
969	+	0	0	0	0	-	-	-	-
976	+	+	+	-	-	-	-	-	-
1011	+	?	?	0	0	0	0	0	0
1016	+	0	0	0	0	0	0	0	0
1017	+	0	0	0	0	0	0	0	0
1:5,000 Dilution of Histamine									
969	+	0	0	0	0	-	-	-	-
976	0	0	+	-	-	-	-	-	-
1060	+	+	+	0	0	-	-	-	-
1061	+	+	+	0	0	-	-	-	-
1065	+	-	+	0	0	0	0	0	0
1066	+	-	+	0	0	0	0	0	0
1:3,000 Dilution of Histamine									
969	+	+	0	0	0	-	-	-	-
976	+	0	0	-	-	-	-	-	-
1060	+	+	+	0	0	-	-	-	-
1061	+	+	0	0	0	-	-	-	-
1065	+	-	+	0	+	0	0	0	0
1066	+	-	+	+	0	0	0	0	0
1:1,000 Dilution of Histamine									
784	+	+	+	+	+	+	+	+	+
945	+	+	+	0	+	+	+	+	0
946	+	+	+	+	+	+	+	+	+
969	+	+	+	+	+	-	-	-	-
976	+	+	+	-	-	-	-	-	-
804	+	+	+	+	+	+	+	+	+
1060	+	+	-	+	+	-	-	-	-
1061	+	+	-	+	+	-	-	-	-

EXPERIMENTAL

OBSERVATIONS ON THE LOCALIZATION AND THE CONCENTRATION OF TRYPAN BLUE IN AREAS OF SKIN INJECTED WITH HISTAMINE

A group of rabbits are injected intradermally with a series of different dilutions of histamine at the following intervals before 10 c.c. of trypan blue are injected intravenously: six hours, five hours, four hours, three hours, two hours, one hour, thirty minutes, fifteen minutes, and immediately. The concentration of histamine injected and the location of the dye in the skin are shown in Table I. It is evident from these data that trypan blue localizes and

concentrates for less than thirty minutes in areas of skin previously injected with dilutions of histamine less than 1:3,000. When a 1:1,000 dilution of histamine is injected, the dye may localize and concentrate in areas of skin injected as long as five to six hours previous to the injection of the dye. Some of these rabbits are injected with only a single dilution of the histamine.

The skin at the site of the injection of the histamine usually becomes blanched immediately following an intradermal injection. This color persists for varying periods of time. The skin around this blanched area becomes hyperemic and apparently edematous. The macroscopic size of the skin lesion is influenced by the concentration of the histamine. The lesions may persist for six hours when a 1:1,000 dilution of histamine is injected. There is only an insignificant amount of blanching when a 1:10,000 dilution of histamine is injected. The hyperemia persists for only a short time.

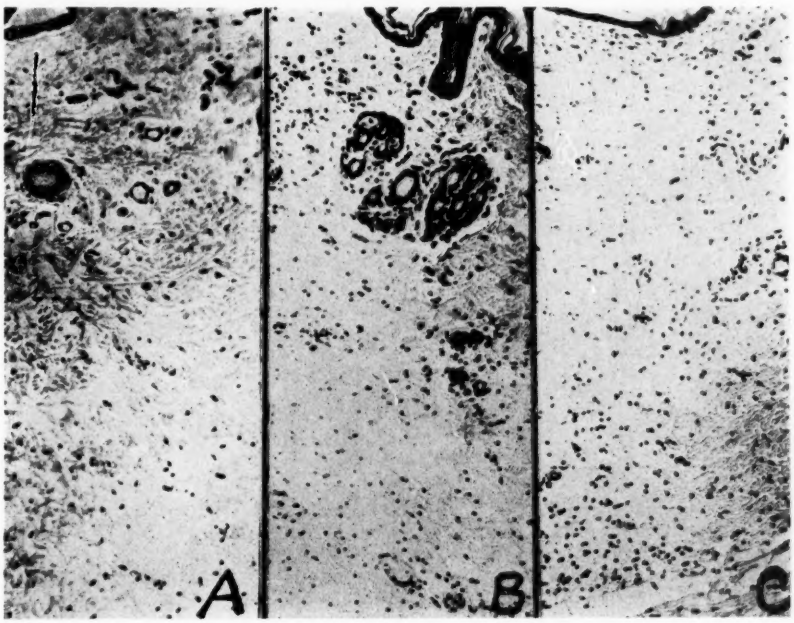

Fig. 1.—*A*, The subcutaneous tissue is slightly edematous. How much of the edema results from the fluid injected and how much from the effect of the histamine on the capillary walls cannot be determined histologically. This rabbit was injected intradermally with 0.2 c.c. of a 1:1,000 dilution of histamine phosphate for ten minutes before this section was removed.

B, In addition to some edema in the subcutaneous tissue there are few polymorphonuclear leucocytes. These are present especially around the small blood vessels. This is the same rabbit as shown in *A*. Two-tenths cubic centimeter of the 1:1,000 dilution of histamine phosphate was injected intradermally five hours before this section was removed.

C, Leucocytes and edema are present in the subcutaneous tissue. This is the same rabbit as shown in *A* and *B*. Two-tenths cubic centimeter of the 1:1,000 dilution of histamine phosphate was injected intradermally eight hours before this section was removed.

Trypan blue stains a ring of tissue blue around the blanched zone at the site of the intradermal injections. This dark blue zone may measure as much as 1 cm. The blue color then gradually fades as it extends peripherally. The entire area of skin becomes more or less uniformly blue when the most dilute solutions of histamine are injected. Trypan blue may fail to localize and to concentrate in areas of skin injected with a 1:3,000, 1:5,000, 1:8,000, and

1:10,000 dilution of histamine, although the skin is hyperemic. This occurs when the dye is given either thirty minutes or longer following the injection of the histamine.

HISTOLOGIC STUDIES UPON THE AREAS OF SKIN INJECTED WITH HISTAMINE

Sections of skin are removed from 5 rabbits injected with 0.2 c.c. of a 1:1,000 dilution of histamine at the following intervals before the animals are killed: 0 minutes, fifteen minutes, thirty minutes, one hour, two hours, three hours, four hours, five hours, and six hours. Sections of skin are also removed from 3 other rabbits injected with 0.2 c.c. of a 1:8,000 dilution of histamine at corresponding intervals before death.

Fig. 2.—Small, focal areas of degeneration occur in the fibers of the cutaneous maximus muscle following the intradermal injection of 0.2 c.c. of a 1:1,000 dilution of histamine phosphate. Polymorphonuclear leucocytes infiltrate the area about the necrotic tissue. The histamine was injected five hours before this section was removed.

The histologic changes are essentially the same when a 1:8,000 and a 1:1,000 dilution of histamine is injected. There may be some difference in the extent of the lesion, but this factor is difficult to determine from these sections. It is also difficult to separate the effect of the volume of fluid injected from the edema fluid that results from the direct effect of the histamine on the capillaries. Polymorphonuclear leucocytes are present in the extravascular tissue usually within an hour following the injection of the histamine. The number of these cells apparently increases during the following four to five hours. After this time apparently the number of leucocytes decreases. Leucocytes usually infiltrate the corium and the subcutaneous tissues down to the cutaneous maximus muscle (Fig. 1). Sometimes there is a pink stain material in the subcutaneous tissue. It is considered to be coagulated serum.

There are small focal areas of degeneration in the cutaneous maximus muscle. It is impossible to determine accurately the time at which this lesion begins; however, the sections removed two hours after the histamine is injected

show unquestionable necrosis. This lesion is characterized by a swelling of the muscle fiber and a loss of the striations. The area appears glossy and stains deeply with eosin. In some of the sections the muscle fibers are broken and the margins are irregular. Polymorphonuclear leucocytes are present in large numbers around such muscle fibers (Fig. 2).

Four rabbits are injected subcutaneously with 0.2 c.c. of a 1:1,000 dilution of histamine at the following intervals before they are killed and the sections are removed: seven, six, five, four, three, two, and one hours. An attempt is made to inject the histamine into the cutaneous maximus muscle in these animals. This technique is unsatisfactory. The sections from this material show the same histologic changes as those observed in the other animals, except there is a more extensive lesion in the muscle of these animals than there is in the former group of rabbits.

EFFECT OF HISTAMINE PHOSPHATE ON THE NUMBER OF CIRCULATING LEUCOCYTES IN THE RABBIT

The data from these counts are given in Table II. The number of leucocytes in the circulating blood increases during the seven hours following the intravenous injection of histamine. The number of leucocytes in the circulating blood, however, returns to within the range of normal twenty-four hours following the injection of the histamine.

TABLE II

EFFECT OF HISTAMINE PHOSPHATE ON THE NUMBER OF CIRCULATING WHITE BLOOD CELLS IN THE RABBIT

RABBIT NO.	9:00 A.M.	10:00 A.M.	11:00 A.M.	1:00 P.M.	2:00 P.M.	4:00 P.M.	8:00 A.M.	1:00 P.M.
914	9450	21600	17200	26550	11800	14350	16350	10450
915	7750	13300	19650	14150	14450	15500	14500	12600
916	10350	15450	16050	33300	19900	21350	22200	17950
917	14200	15750	22600	30400	22100	13650	9950	14500
1012	11450	12500	40800	41800	47400	13500		
1013	7800	29150	27200	12900	26250	12350		
1014	7600	17150	19800	13700	17850	17800		
1015	10150	6000	14600	25000	19100	14600		
Means	9843	16362	22212	24725	22356	15387	15750	13875
Differences in mean count*		+6519	+12369	+14882	+12513	+5544		
Value of t_1†		2.56	3.98	3.90	3.14	4.26		
Value of t_2‡				5.25	5.87	2.68		

*Differences in the mean leucocyte counts and the value of t for various hours.
t must equal 2.145 or more for the increase to be statistically significant.
Histamine injected at 9:00 A.M. immediately following the blood count.

†t_1 Value of t when the experimental animals are compared hour to hour—14 degrees of freedom.

‡t_2 Value of t when experimental animals are compared with 32 control animals—38 degrees of freedom.

The values of t are calculated for these samples of 8 rabbits in order to compare the counts at 9:00 A.M., 10:00 A.M., 11:00 A.M., 1:00 P.M., 2:00 P.M., and 4:00 P.M. of the day of injection; and counts are made again at 8:00 A.M. and 1:00 P.M. on the following day. t must equal for 14 degrees of freedom 2.145 or more if the increase in the mean leucocyte count is significant. The

mean counts at the specified hours and the values of t are given in Table II. The mean leucocyte counts of the experimental animals are also compared with the mean leucocyte counts of 32 control animals, and the values of t for these comparisons are also shown in Table II. For 38 degrees of freedom t must equal 2.02 for the differences to be statistically significant.

DISCUSSION

It is evident from this study that histamine phosphate, when injected intradermally in the rabbit, acts as a chemotactic substance. This observation is confirmatory of the studies of Wolf[5] and Findlay;[6] however, it does not support the opinion expressed by Bloom[3] and Grant and Wood[4] as to the effect of histamine on the attraction of leucocytes.

The presence of necrosis in the cutaneous maximus muscle, following the intradermal injection of 0.2 c.c. of a 1:1,000 and 1:8,000 dilution of histamine, indicates that this substance may produce not only an increase in capillary permeability, but also a severe injury that culminates in necrosis. Because of this necrosis it becomes more difficult to ascertain the role of histamine in inflammation.

Trypan blue following an intravenous injection localizes and concentrates in areas of skin previously injected with histamine. The localization and the concentration of trypan blue in these areas are influenced by both the interval between the injection of the histamine and the injection of the dye, and the concentration of histamine. The skin, where 0.2 c.c. of a 1:1,000 dilution of histamine is injected, becomes blue within a period of five minutes when the dye is injected intravenously as long as six hours after the intradermal injection of the histamine. In contrast to this the skin does not show a greater concentration of dye than that in the untreated area, when an interval of thirty minutes elapses between the injection of a 1:10,000 dilution of histamine and the intravenous injection of the trypan blue. These observations suggest that histamine, when injected intradermally in the rabbit, produces a disturbance in the metabolism of the regional cells. Trypan blue following an intravenous injection localizes and concentrates in this area of skin only during the time that this abnormal metabolic process is present. The cells may return to the "so-called normal" very quickly, as indicated by the failure of trypan blue to localize and to concentrate in the skin when a 1:10,000 dilution of histamine is injected thirty minutes before the dye is given. The cells remain abnormal for at least six hours when a 1:1,000 dilution of histamine is injected. This interval during which trypan blue may localize and concentrate in areas of skin previously injected with histamine may be influenced by the total quantity of histamine given to the animal.

A specific period during which trypan blue localizes and concentrates in an area is the same phenomenon as previously observed with a group of irritants and a variety of circulating substances. Trypan blue, India ink, a virus, staphylococci, and staphylococcus antitoxin each localizes and concentrates in the skin only within a period of three hours following the local application of xylol.[11] When staphylococci are injected intradermally and trypan blue is given intravenously, the largest amount of the dye localizes in the area where

the bacteria are injected within a period of three hours previous to the injection of the dye.[17] In a discussion of these observations it has been suggested that "the process in inflammation referred to as increased capillary permeability represents a phenomenon well recognized in vital staining. The staining results from a metabolic disturbance in the epithelial cells, connective tissue cells, and endothelial cells of the small blood vessels."[18]

It is of interest to know that the polymorphonuclear leucocytes concentrate in areas of skin injected intradermally with histamine for a much longer period than that in which trypan blue localizes and concentrates in the same area. It has been pointed out in other studies that "the dye may localize and concentrate in tissue when there are no polymorphonuclear leucocytes and fail to localize and to concentrate when the subepithelial tissues are diffusely infiltrated with leucocytes."[18]

The data in this experiment show that there is a statistically significant increase in the leucocyte counts of rabbits injected intravenously with 0.4 c.c. of a 1:1,000 dilution of histamine phosphate. This increase occurs within one hour of the time of injection and continues for at least seven hours. These data are obtained by comparing the counts of the experimental animals with the counts from a group of 32 normal rabbits.

The demonstration of a leucocytosis following the injection of histamine phosphate corresponds to the observations of Moon.[2] He finds that histamine produces a leucocytosis in cats, monkeys, and human beings. Menkin,[8] however, considers that histamine is essentially inactive for producing an increase in the leucocytic level in the blood of both dogs and rabbits.

It is obvious that the phenomena that occur in the local areas of inflammation and in the circulating blood are complex. These experimental observations with histamine may be an index of some of these changes; however, one must be careful in interpreting them, since there appears to be a variety of unrelated substances, each of which may produce an increase in capillary permeability and a localization of leucocytes in the skin of the rabbit.

SUMMARY

Histamine phosphate, when injected intradermally in the rabbit, produced a change in the tissue cells that permits the localization and the concentration of trypan blue in the local area. This localization and concentration of trypan blue are influenced by both the concentration of histamine and the interval between the intradermal injection of the histamine and the intravenous injection of the dye.

Polymorphonuclear leucocytes localize in the subcutaneous tissues of the rabbit following an intradermal injection of histamine phosphate.

Necrosis occurs in the fibers of the cutaneous maximus muscle following the intradermal injection of histamine phosphate.

The number of white blood cells increases in the circulating blood following an intravenous injection of histamine phosphate. The number, however, returns to normal within a period of twenty-four hours.

I am greatly indebted to Dr. F. L. Roberts, Professor of Preventive Medicine, University of Tennessee, for his assistance in the statistical problems concerned in this study.

REFERENCES

1. Lewis, Thomas: The Blood Vessels of the Human Skin and Their Responses, London, 1927, Shaw & Sons.
2. Moon, Virgil H.: Shock and Related Capillary Phenomena, New York, 1938, Oxford University Press.
3. Bloom, W.: Histamine as an Inflammatory Agent, Bull. Johns Hopkins Hosp. **33**: 185, 1922.
4. Grant, R. T., and Wood, J. E.: Histamine and Leucocytes Emigration, J. Path. & Bact. **31**: 1, 1928.
5. Wolf, E. P.: Experimental Studies on Inflammation; Influence of Chemicals on Chemotaxia of Leucocytes in Vitro, J. Exper. Med. **34**: 375, 1921.
6. Findlay, G. M.: Histamine and Infection, J. Path. & Bact. **31**: 633, 1928.
7. Best, C. H., and McHenry, E. W.: Histamine, Physiol. Rev. **11**: 371, 1931.
8. Menkin, Valy: Dynamics of Inflammation, New York, 1940, The Macmillan Co.
9. Menkin, Valy: A Note on the Differences Between Histamine and Leukotaxine, Proc. Soc. Exper. Biol. & Med. **40**: 103, 1939.
10. Rigdon, R. H.: Capillary Permeability in the Skin of the Rabbit, Proc. Soc. Exper. Biol. & Med. **42**: 43, 1939.
11. Rigdon, R. H.: Capillary Permeability in Areas of Inflammation Produced by Xylene, Arch. Surg. **41**: 101, 1940.
12. Rigdon, R. H.: Localization of Staphylococci in Areas of Inflammation Produced by Xylene, Arch. Surg. **41**: 879, 1940.
13. Rigdon, R. H., and Haynes, Anne: Observations on Capillary Permeability and Inflammation in the Skin of Sensitized Rabbits, J. Lab. & Clin. Med. **27**: 598, 1942.
14. Rigdon, R. H., and Wilson, H.: Capillary Permeability and Inflammation in Rabbits Given Heparin, Arch. Surg. **43**: 64, 1941.
15. Rigdon, R. H., Miles, Robert M., and Bland, Richard P.: The Effect of Low Blood Pressure Upon Capillary Permeability and Inflammation in the Skin of Rabbits, Surgery **12**: 302, 1942.
16. Fisher, R. A., and Yates, F.: Statistical Tables, London, 1938, Oliver & Boyd.
17. Rigdon, R. H.: Observations on Capillary Permeability in Areas of Inflammation Produced by Staphylococci, Surgery **9**: 436, 1941.
18. Rigdon, R. H.: Relation of Capillary Permeability to Inflammation, South. M. J. **34**: 292, 1941.

CLINICAL CHEMISTRY

CHOLESTEROL STUDIES IN THE AGED*

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NUMEROUS investigators[1-11] have studied the total cholesterol concentration and its partition, that is, the ratio of the free cholesterol, or the cholesterol esters, to the total cholesterol in the blood of persons below 60 years of age. Comparatively scant attention, however, has been paid to the cholesterol partition in the blood of normal persons past 60 years of age. A difference of opinion also exists as to whether advancing age affects the cholesterol values. In 1923 Parhon and Parhon[12] stated that the total cholesterol concentration in whole blood increased slightly with age. In 1924 Bing and Heckscher[13] found that the blood lipids were relatively low in youth and increased with advancing age. These observers stated that the upper and lower limits for the total cholesterol in the blood of normal aged persons were 260 and 125 mg. per 100 c.c., respectively. On the other hand, Page, Kirk, Lewis, Thompson, and Van Slyke[4] found that in a study of the lipids in the plasma of males of all ages there was no regular important change in any blood lipid fraction with advancing years. Similarly, Hunt[11] stated that the age of the patient does not affect the blood cholesterol values. It was, therefore, thought of interest to make a study of the blood cholesterol and its partition in the serum and whole blood of normal aged males and females.

EXPERIMENTAL STUDY

The subjects chosen for this study comprised 76 males and 74 females. All were housed in the "Home" division of the institution, all were ambulatory, and all were on the normal institutional diet. Their ages ranged from 62 to 104 years and were distributed as outlined in Table I. The mean ages were found to be 77.1 years for males and 76.2 years for females.

Determinations were made of total and esterified cholesterol in both serum and in whole blood, using the method of Bloor and Knudson.[7] All determinations were made after a fasting period of at least eight hours in order to rule out any temporary lipemia due to ingestion of fatty materials. The free cholesterol was computed in each case by subtracting the esterified cholesterol from the total cholesterol, and this value, divided by the total cholesterol, gave the fraction existing in the free state.

Table II shows the distribution of the total cholesterol concentration in milligrams per 100 c.c. for both whole blood and serum for both sexes. Table III shows the distribution of the free cholesterol percentages for both whole blood and serum for both sexes.

*From the Hospital and Home of the Daughters of Jacob, New York.
This study was aided by a grant from the Paul Hirsch Fund.
Received for publication, June 1, 1942.

The values for the total cholesterol concentration in the blood of the male subjects in our study ranged from 180 to 340 mg. per 100 c.c. in serum, and from 158 to 316 mg. per 100 c.c. in whole blood. The range of cholesterol concentration in whole blood for females was from 149 to 329 mg. per 100 c.c., and in serum the lower and upper limits were 168 and 320 mg. per 100 c.c., respectively.

TABLE I

AGE-FREQUENCY DISTRIBUTION OF SUBJECTS STUDIED

AGE GROUP (YEARS)	MALES	FEMALES	TOTAL
60-65	4	0	4
65-70	8	14	22
70-75	19	19	38
75-80	21	25	46
80-85	12	10	22
85-90	7	5	12
90-95	5	0	5
95-100	0	0	0
100-105	0	1	1

The free cholesterol in males was between 16 and 64 per cent in serum and between 14 and 75 per cent in whole blood. The free sterol for females showed a variation between 17 and 67 per cent in serum and between 11 and 75 per cent for whole blood. The average total cholesterol values in whole blood were computed to be 219 mg. per 100 c.c. for males and 227 mg. per 100 c.c. for females, and in serum the average total cholesterol values were found to be 246 mg. per 100 c.c. for males and 232 mg. per 100 c.c. for females. The average value for free cholesterol in whole blood was 39 per cent for males and 50 per cent for females, while in serum the average free cholesterol was 37 per cent for males and 42 per cent for females. The over-all average for total cholesterol in whole blood was 223 mg. per 100 c.c.; with the free sterol amounting to 46 per cent. Averaging all the values for serum gave a total cholesterol of 238 mg. per 100 c.c. of which 39 per cent existed in the free state.

COMMENT

The total cholesterol and cholesterol partition were studied in a group of 150 so-called normal aged persons. According to Todd and Sanford,[15] the normal limits for total cholesterol estimated by the Bloor method were 165 to 200 mg. per 100 c.c. for plasma or serum with 30 per cent as the maximum normal free sterol. Bloor and Knudson[7] found that the total concentration of cholesterol was from 180 to 200 mg. per 100 c.c., "little difference being noted between the results for whole blood and serum if the normal ratio of esters to free sterol is maintained." They further stated that in serum the maximum normal percentage of free sterol encountered was about 40 per cent, and in whole blood the free cholesterol ran to a maximum of 33 per cent. Pickhardt, Bernhard, and Kohn[11] regarded 30 per cent as the maximum normal percentage of free sterol in serum. Bodansky and Bodansky[2] stated that the concentration of total cholesterol in plasma was between 160 and 200 mg. per 100 c.c., with the free sterol around 30 per cent with very

slight variation. On the basis of these and other studies,[1, 4, 6, 10] the maximum normal concentration of total cholesterol in either serum or whole blood may be regarded as 200 mg. per 100 c.c., with a maximum of 30 to 40 per cent existing as the free sterol.

TABLE II

FREQUENCY DISTRIBUTION FOR TOTAL CHOLESTEROL

TOTAL CHOLESTEROL MG./100 C.C.	WHOLE BLOOD		SERUM		TOTAL
	MALES	FEMALES	MALES	FEMALES	
140-160	2	2	0	0	4
160-180	9	7	0	3	19
180-200	14	9	4	6	33
200-220	6	10	3	4	23
220-240	8	2	5	5	20
240-260	2	5	3	2	12
260-280	5	3	2	1	11
280-300	4	3	4	1	12
300-320	4	0	1	4	9
320-340	0	3	0	1	4
340-360	0	0	1	0	1
360-380	0	2	0	0	2

TABLE III

FREQUENCY DISTRIBUTION FOR PERCENTAGE FREE CHOLESTEROL

FREE CHOLESTEROL %	WHOLE BLOOD		SERUM		TOTAL
	MALES	FEMALES	MALES	FEMALES	
10-15	1	1	0	0	2
15-20	1	0	4	1	6
20-25	3	1	1	1	6
25-30	6	2	3	5	16
30-35	5	2	5	2	14
35-40	6	3	1	1	11
40-45	6	9	2	5	22
45-50	6	3	3	3	15
50-55	7	9	2	4	22
55-60	1	2	2	3	8
60-65	4	8	1	1	14
65-70	3	2	0	1	6
70-75	2	3	0	0	5
75-80	1	2	0	0	3

In this study, as can be seen from Tables II and III, when whole blood was used, 29 males and 28 females, or 57 per cent of the subjects investigated, had a total cholesterol concentration of more than 200 mg. per 100 c.c.; 30 males and 38 females, or 68 per cent, had a free cholesterol of more than 40 per cent. When serum was employed, 19 males and 18 females, or 74 per cent of the subjects studied, had a total cholesterol of more than 200 mg. per 100 c.c.; 10 males and 17 females, or 54 per cent, showed a free cholesterol in excess of 40 per cent.

The question now arises as to whether the comparatively high total and free cholesterol in the normal aged person has any significance. Are these increased cholesterol values a natural concomitant of old age? The persons in this study did not present any clinical evidence of liver dysfunction, biliary tract disease, diabetes, or renal impairment, which conditions are often accompanied by a hypercholesterolemia and a disturbed cholesterol partition. Arteriosclerosis is a factor that must be taken into consideration in any per-

son past 50 years of age. Kilduffe[16] stated that increased cholesterol values are often associated with arteriosclerosis. Leary[17] thought that arteriosclerosis is probably the result of abnormal cholesterol metabolism. Best and Taylor[18] stated that one of the means by which arteriosclerosis could be produced in rabbits was by feeding diets high in cholesterol. They also claimed that the administration of cholesterol is one of the most certain means of producing excessive deposition of neutral fats in the liver, with a resulting accumulation of cholesterol esters.

SUMMARY

Cholesterol studies were made in 150 normal aged persons. Of the 100 persons in whom whole blood was studied, 57 per cent had a total cholesterol concentration of more than 200 mg. per 100 c.c., and 68 per cent showed free cholesterol of more than 40 per cent. Of the 50 persons in whom serum was used, 74 per cent had a total cholesterol concentration of over 200 mg. per 100 c.c., and 54 per cent had an excess of 40 per cent free cholesterol.

We wish to thank Dr. Samuel Seidenberg for his cooperation.

REFERENCES

1. Campbell, J. M. H.: Cholesterol in Health and Disease, Quart. J. Med. **18**: 393, 1925.
2. Bodansky, M., and Bodansky, O.: The Biochemistry of Disease, New York, 1940, The Macmillan Co., page 81.
3. Lichtenstein, L., and Epstein, E. Z.: The Blood Lipoids in Nephrosis and Chronic Nephritis With Edema, Arch. Int. Med. **47**: 122, 1931.
4. Page, I. H., Kirk, E., Lewis, E. H., Jr., Thompson, W. R., and Van Slyke, D. D.: Plasma Lipids of Normal Men at Different Ages, J. Biol. Chem. **111**: 613, 1935.
5. Sperry, W. M.: The Concentration of Total Cholesterol in the Blood Serum, J. Biol. Chem. **117**: 391, 1937.
6. Stitt, E. R., Clough, P. W., and Clough, M. C.: Practical Bacteriology, Hematology, and Parasitology, Philadelphia, 1939, P. Blakiston's Son & Co., page 677.
7. Bloor, W. R., and Knudson, A.: Cholesterol and Cholesterol Esters in Human Blood, J. Biol. Chem. **29**: 7, 1917.
8. Muller, G. L.: The Cholesterol Metabolism in Health and Anemia, Medicine **9**: 119, 1930.
9. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1931, Williams & Wilkins Co.
10. Cantarow, A., and Tromper, M.: Clinical Biochemistry, Philadelphia, 1939, W. B. Saunders Co., page 172.
11. Pickhardt, O. C., Bernhard, A., and Kohn, I. L.: The Significance of the Cholesterol Partition of the Blood Serum in Surgery of the Gall Bladder, Ann. Surg. **110**: 701, 1939.
12. Parhon, J. C., and Parhon, M.: L'hypercholesterinemie de la viellesse, Compt. rend. Soc. de biol. **88**: 231, 1923.
13. Bing, H. I., and Heckscher, H.: II. Mitteilung über die Fettmenge des Blutes bei normalen Menschen, Biochem. Ztschr. **83**: 144, 1924.
14. Hunt, H. M.: Cholesterol in Diabetes, New England J. Med. **201**: 660, 1929.
15. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, Philadelphia, 1939, W. B. Saunders Co., page 783.
16. Kilduffe, R. A.: Clinical Interpretation of Blood Examinations, Philadelphia, 1931, Lea & Febiger, page 495.
17. Leary, T.: Atherosclerosis and Cholesterol Metabolism, Bull. New York Acad. Med. **17**: 887, 1941.
18. Best, C. H., and Taylor, G.: The Physiological Basis of Medical Practice, Baltimore, 1938, William Wood & Co., page 211.

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SULFONAMIDE SOLUBILITY IN UREA*

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SULFONAMIDE hematuria, well recognized in both clinic and laboratory, is closely associated with mechanical traumatic effects following precipitation of insoluble acetylated sulfanilyl derivatives within the urinary tract. Recent studies by Curtis and Sobin[1] have shown that the solubility of *acetylsulfapyridine* and *acetylsulfathiazole* is considerably enhanced by an alkaline pH and by increasing amounts of urea in solution. Both of these factors may well explain the difference in solubility of these substances in water and urine. The peptizing effect of urea is also seen with other more simple chemical substances.[2]

Analysis of the precipitated material found in the urinary tract of man and animals has shown that in sulfapyridine-treated cases more free drug is present than in the sulfathiazole-treated cases.[3-6] To determine a possible mechanism for the differences in free sulfonamides in uroliths, the solubility of the *free acids* of sulfapyridine, sulfathiazole, and sulfadiazine in varying concentrations of urea have been studied.

It was further hoped that the solvent effect of urea on these sulfonamides might be sufficient to allow the preparation of a soluble form for parenteral use without the attendant dangers of a high pH of the alkaline salts.

METHOD

Urea solutions of varying concentrations from 0.1 to 10 per cent were incubated at 37° C. with an excess of sulfapyridine, sulfathiazole, and sulfadiazine, shaken at intervals, and filtered through two thicknesses of Whatman No. 42 filter paper. After appropriate dilution the free sulfonamide was determined by the method of Bratton and Marshall,[7] using the Evelyn colorimeter and a No. 540 filter.

RESULTS

The results of this series of observations are shown graphically in Fig. 1. Solubility in milligrams per 100 c.c. is plotted on the ordinates, and concentration of urea in per cent on the abscissae. The order of increasing solubility is sulfadiazine, sulfapyridine, and sulfathiazole. The order of increasing solvent effect of urea is the same as their solubilities.

DISCUSSION

It has been previously shown that the solubility of acetylsulfathiazole is slightly increased by urea, and that of acetylsulfapyridine is more definitely increased.[1] In neither case is the total solubility in 10 per cent urea solution above 40 mg. per 100 c.c., as contrasted with the values of 133.0 mg. per 100 c.c. for sulfathiazole and 73.3 mg. per 100 c.c. for sulfapyridine in similar 10 per cent urea solution. The difference in solubility between the free acids them-

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Received for publication, June 18, 1942.

selves and between the free acid and their acetylated compounds is sufficient to explain the concentration of sulfapyridine and sulfathiazole in analyzed uroliths.

The solubility of sulfadiazine is quite low, and in 10 per cent urea the maximum solubility was found to be 31.5 mg. per 100 c.c. This figure approximates the solubility of acetylsulfapyridine and acetylsulfathiazole, and re-emphasizes a potential danger in the use of this drug—already experienced clinically.

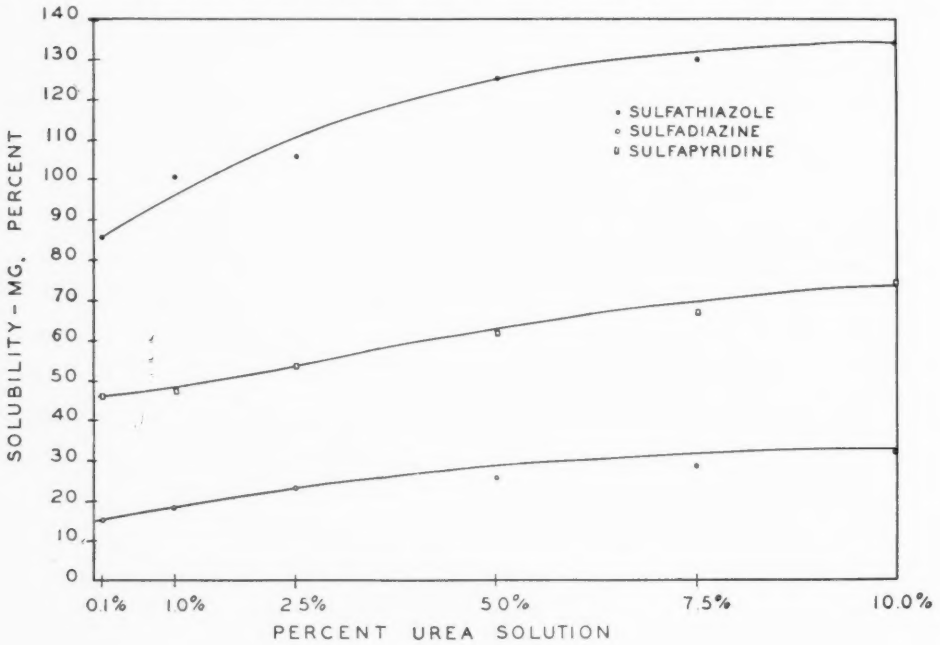

Fig. 1.

The solvent effect of urea on these sulfonamides is not of sufficient degree to be adaptable to parenteral sulfonamide therapy.

CONCLUSIONS

1. Urea increases the solubility of sulfathiazole, sulfapyridine, and sulfadiazine.

2. The extent of such increase in solubility may explain some related problems of renal precipitation of sulfonamides.

3. Sulfonamide's solubility in urea-sulfonamide solutions is not sufficient to warrant its use in parenteral therapy.

REFERENCES

1. Curtis, A. C., and Sobin, S. S.: The Solubility of Acetylsulfapyridine and Acetylsulfathiazole in the Urine, Ann. Int. Med. **15**: 884, 1941.
2. Medes, G.: Solubility of Calcium Oxalate and Uric Acid in Solutions of Urea, Proc. Soc. Exper. Biol. & Med. **30**: 281, 1932.
3. Gross, P., Copper, F. B., and Lewis, M.: Urinary Calculi Caused by Sulfapyridine, Urol. & Cutan. Rev. **43**: 299, 1939.
4. Gross, P., Copper, F. B., and Scott, R. E.: Urolithiasis Medicamentosa, Urol. & Cutan. Rev. **44**: 205, 1940.
5. Sadusk, J. F., Jr., Waters, L., and Wilson, D.: Anuria Due to Sulfapyridine Calculi, J. A. M. A. **115**: 1968, 1940.
6. Lowenberg, S. A., Sloane, N. G., and Chodoff, P.: Sulfathiazole Urinary Calculi, J. A. M. A. **115**: 2069, 1940.
7. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**: 537, 1939.

LABORATORY METHODS

GENERAL

A STUDY OF THE PHENOMENON OF ERYTHROCYTE SEDIMENTATION*

III. Anticoagulation as a Technical Source of Variations

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IN A PREVIOUS publication a critical survey of considerable literature dealing with sedimentation was presented.[1] In another publication[2] a reliable technique for measuring sedimentation, a more practical "field technique" and evidence of the reproducibility and limitations of each were advanced. This paper includes evidence supporting my choice of the kind and amount of anticoagulant used, as well as the limitations of this choice and variations due to other kinds and quantities of anticoagulants.

The choice of a suitable means of anticoagulation for sedimentation studies in equine blood has been a perplexing problem. Many different concentrations of several kinds of anticoagulants were attempted, examples of results of which are to follow. Defibrination was also attempted but, because of varying degrees of hemolysis and somewhat variable results, was discarded. It is realized that Fahraeus[3] employed this method of anticoagulation quite extensively with horse blood but used as a criterion of sedimentation a single reading at the end of one hour, a method of reading which because maximum sedimentation has passed will yield somewhat comparable results in practically all samples of equine blood having fairly close volume percentages. Fahraeus admitted that his method did not in reality measure sedimentation.

In defibrinated samples sedimentation is slower than in samples with added anticoagulants. In samples in which the process of defibrination caused the least cell damage, and plasma, although darker than anticoagulant plasma, was not reddish, volume percentages were closer to those of anticoagulant samples. Even in defibrinated samples where plasma was only slightly darker and volume percentages were quite uniform, sedimentation was much slower, as for example with potassium and ammonium oxalated aliquots of the same blood sample:

Aliquot No.	M_5‡	t_{M_5}‡	$\angle^s M_5$	V%	Plasma
1	26 mm.	10-15 min.	30° 20′	35.5	Clear
2	25 mm.	10-15 min.	31° 30′	36	Clear
3	26 mm.	10-15 min.	30° 20′	36-	Clear

*From a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Ohio State University, June, 1941.

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Received for publication, April 3, 1942.

‡*Definitions:*

M_5 = Maximum five-minute descent in millimeters of the line of demarcation between cells and plasma.

t_{M5} = Times in minutes between which M_5 occurs. Since with the photographic apparatus one minute is the equivalent of 3 mm., this time-distance ratio is retained for the "field" technique.

With defibrinated aliquots of the same blood sample as the foregoing:*

Aliquot No.	M_5	$^{t}M_5$	$\angle^{s}M_5$	V%	Plasma
4	18 mm.	{ 5-10 min. {10-15 min.	40° 20′	34	Darker
5	19 mm.	10-15 min.	38° 50′	33.7	Darker
6	20 mm.	10-15 min.	37° 20′	33.7	Darker

Photographic recordings of sedimentation of aliquots of the same sample of blood, with hemolysis at a minimum, are like Fig. 1.

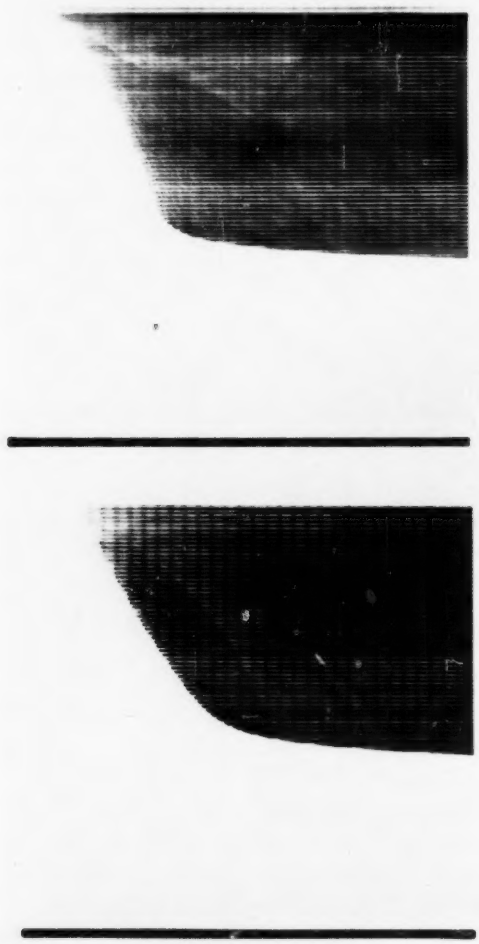

Fig. 1.—Sedimentation curves with the ammonium and potassium oxalate mixture (top) and defibrination (bottom).

*_Definitions_ (cont'd):

$\angle_{M5}$ = Angle opposite the horizontal side of a right triangle having M_5 as the perpendicular side and five minutes, or 15 mm., as the horizontal side.

V% = Volume per cent of packed cells.

em = The perpendicular distance from the estimated point of change between the vertical flexion and horizontal flexion of the photographically recorded sigmoid curve of sedimentation of a sample and the top line of the photograph representing 100 mm. of blood column height, i.e., estimated "fall" in millimeters at the point of change.

t_{em} = Time in minutes between the time of filling of the Wintrobe tube and the time that the point of change between the vertical flexion and horizontal flexion of the photographically recorded sigmoid curve of sedimentation of a sample occurs (one minute = 3 mm.)

$\angle_{g}$ = The least angle between the steepest slope of the sigmoid curve of sedimentation of a sample and perpendicularity.

With potassium and ammonium oxalate:

Ex. No.	em*	t_{em}*	$\angle^{s}_{g}$*	M_5	t_{M_5}	$\angle^{s}M_5$	V%
1	31 mm.	9 min.	20°	30 mm.	5-10 min.	27°	34

With defibrination:

2	19 mm.	8 min.	29° 10′	25 mm.	5-10 min.	31° 20′	33.3

It was found that, if careful measuring was employed, similar results could be obtained with similar quantities of anticoagulant. It was felt then that these anticoagulants should each be tested for the most suitable quantity and then these most suitable quantities compared. It was decided to choose that anticoagulant which had the least effect on both sedimentation and volume per cent over the widest range of concentration, and to use a minimal but safe quantity of it, realizing, of course, that there might be some individual differences in the minimal quantity.

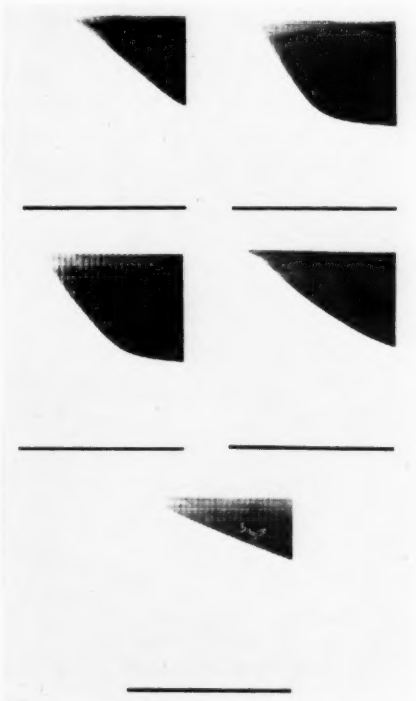

Fig. 2.—Effects of varying quantities of lithium citrate upon sedimentation.

Accurate 2 per cent solutions of the following anticoagulants were prepared in double distilled water: potassium oxalate, sodium oxalate, lithium oxalate, ammonium oxalate, potassium citrate, sodium citrate, lithium citrate, ammonium citrate, and sodium fluoride.

Also a 2 per cent solution of a mixture of 3 parts ammonium oxalate and 2 parts potassium oxalate and a 0.05 per cent solution of heparin containing 110 units per milligram were prepared.

*See footnotes, page 1570, for definitions.

Collecting vials (0.5 ounce) previously graduated at 5 c.c. were prepared with the following concentrations of dry anticoagulants by introducing the proper quantity of the afore-mentioned solutions and allowing the water to evaporate in an incubator:

2, 4, 8, 12, 16, 20, 24 mg. for all the oxalates.
4, 8, 12, 16, 20, 24 mg. for the citrates and sodium fluoride.
5.5, 11.0, 16.5, 22.0, 27.5, 55.0 units of heparin.

Blood was collected as previously described[2] and divided into the 5 c.c. aliquots for each set. Blood and anticoagulant were vigorously shaken for two minutes by hand. While no specific check was made of the completeness of the mixture of anticoagulant and blood, it is felt that, since the anticoagulant was disbursed as much as possible in the collecting vial to facilitate its solution, error in this respect is probably at least minimized. Of course, previous to determination of sedimentation, each aliquot was uniformly agitated in the mechanical shaker previously described.[2]

Although there were minor variations in different samples, results which follow show at least trends. (In the following tabulated data *C* stands for clotting to the extent that measurements of sedimentation could not be made; *c* for evidences of small clots; *f* for what was thought to be fibrin precipitation observed in the plasma; *d* for slightly darker plasma; and *h* for plasma with a definite reddish cast.)

Anticoagulant—potassium oxalate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^{s}M_5$	V%	Plasma
2	C	–	–	–	–
4	16 mm.c	20-25 min.	43° 50′	41.7	Clear
8	18 mm.	15-20 min.	40° 20′	40.3	Clear
12	17 mm.	15-20 min.	41° 50′	39.5	Clear
16	12 mm.	15-20 min.	50° 30′	38.5	d
20	6 mm.	10-25 min.	67°	37.7	d

Anticoagulant—sodium oxalate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^{s}M_5$	V%	Plasma
2	C	–	–	–	–
4	17 mm.	20-25 min.	41° 50′	39.5	Clear
8	16 mm.	15-20 min.	43° 50′	38	Clear
12	15 mm.	10-15 min.	45°	36.7	Clear
16	9 mm.	5-10 min.	58° 10′	36	d
20	2 mm.	20-25 min.	81° 10′	35.3	d

Anticoagulant—lithium oxalate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^{s}M_5$	V%	Plasma
2	C	–	–	–	–
4	15 mm.	15-20 min.	45°	–	Clear
8	16 mm.	15-20 min.	43° 50′	38	Clear
12	4 mm.	5-25 min.	74° 10′	37 –	d
16	2 mm.	20-25 min.	81° 10′	36.5	d
20	2 mm.	20-25 min.	81° 10′	36 +	d

Anticoagulant—ammonium oxalate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
2	C	–	–	–	–
4	17 mm.	15-20 min.	41° 50′	39.3	Clear
8	18 mm.	15-20 min.	40° 20′	40.5	Clear
12	17 mm.	20-25 min.	41° 50′	41	Clear
16	17 mm.	20-25 min.	41° 50′	42.3	Clear
20	17 mm.	25-30 min.	41° 50′	43	Clear

Anticoagulant—potassium citrate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
4	C	–	–	–	–
8	C	–	–	–	–
12	C	–	–	–	–
16	17 mm.	15-20 min.	41° 50′	36.5	Clear
20	16 mm.	10-20 min.	43° 50′	36.3	Clear
24	15 mm.	15-20 min.	45°	36	Clear

Anticoagulant—sodium citrate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
4	C	–	–	–	–
8	C	–	–	–	–
12	19 mm.c	10-15 min.	38° 40′	37.3	Clear
16	18 mm.	10-15 min.	40° 20′	36.5	Clear
20	13 mm.	15-25 min.	48° 30′	35.7	d
24	7 mm.	5-10 min.	64° 10′	34.5	d

Anticoagulant—lithium citrate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
4	C	–	–	–	–
8	10 mm.	15-20 min.	50° 30′	37.7	Clear
12	18 mm.	10-15 min.	40° 20′	36.5	Clear
16	17 mm.	10-15 min.	41° 50′	34.7	Clear
20	16 mm.	10-15 min.	43° 50′	34	Clear
24	10 mm.	10-15 min.	55° 30′	33	d

Anticoagulant—ammonium citrate:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
4	C	–	–	–	–
8	23 mm.c	15-20 min.	33° 30′	41	Clear
12	21 mm.	15-20 min.	35° 50′	41.3	Clear
16	22 mm.	15-20 min.	34° 40′	41.8	Clear
20	21 mm.	10-15 min.	35° 50′	42.3	Clear
24	20 mm.	10-15 min.	37° 10′	42.8	Clear

Anticoagulant—sodium fluoride:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
4	C	–	–	–	–
8	C	–	–	–	–
12	3 mm.	10-35 min.	77° 50′	34.0	Clear
16	2 mm.	40-45 min.	81° 10′	33.5	Clear
20	1 mm.	10-60+ min.	85° 10′	33	Clear

Anticoagulant—ammonium and potassium oxalate mixture:

Mg./5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
2	C	–	–	–	–
4		(Compared in later experiment)			
8	20 mm.	20-25 min.	37° 10′	37.7	Clear
12	19 mm.	10-15 min.	38° 40′	38.3	Clear
16	19 mm.	15-20 min.	38° 40′	38	Clear
20	18 mm.	15-20 min.	40° 20′	37.7	Clear
24	17 mm.	10-15 min.	41° 50′	37.5	d

Anticoagulant—heparin:

Units/5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$	V%	Plasma
5.5	C	–	–	–	–
11.0	15 mm.e	15-20 min.	45°	42 +	Clear
16.5	19f	15-20 min.	38° 40′	42 +	Clear
22.0	18f	15-20 min.	40° 20′	42.7	Clear
27.5	20 mm.	15-20 min.	37° 10′	42.7	Clear
55.0	16.5 mm.	10-15 min.	42° 50′	42.7	Clear

Anticoagulant—heparin. Repeated on same aliquots:

Units/5 c.c. of blood	M_5	t_{M_5}	$\angle^s M_5$
5.5	C	–	–
11.0	C	–	–
16.5	19.5 mm.f	15-20 min.	38°
22.0	20.5 mm.	10-15 min.	36° 40′
27.5	19.5 mm.	10-15 min.	38°
55.0	16.5 mm.	15-20 min.	42° 50′

From the foregoing data one can see that, in general, sedimentation tends to decrease the more anticoagulant is used. Volume percentages tend to decrease with potassium, sodium, and lithium oxalates and citrates as the amount of anticoagulant is increased. With ammonium oxalate and ammonium citrate the reverse is true. With heparin and the potassium and ammonium oxalate mixture volume percentages tend to remain fairly close. With the ammonium salts, the potassium and ammonium oxalate mixture and heparin, sedimentation values tend to remain close over a wider range of concentrations than with the others. Since the volume percentages with ammonium oxalate and ammonium citrate vary considerably with concentration, they may be considered as less efficient than heparin and the potassium and ammonium oxalate mixture.

Sodium fluoride apparently cannot be used for equine blood.

Although heparin has many good features as an anticoagulant, it also has some which are disappointing. In practice it is less dependable than the potassium and ammonium oxalate mixture, there apparently being a wide variation in the minimal quantity necessary for anticoagulation. With some horse's blood as low as 11 units per 5 c.c. is sufficient for apparently complete anticoagulation, whereas with the blood of others three or four times that amount sometimes is not. Regardless of whether this is the result of poor solubility or not, or whether it is inherent in the action of the preparation itself or not, such variation seems to exist and naturally leads one to be a bit dubious of the results he obtains. The appearance of the plasma often is an aid in judging the degree of anticoagulation, since in equine blood with heparin the degree of what is apparently lipemia

is less than with the other anticoagulants. Any cloudiness of the plasma, where there are particles of various sizes, or a "flaky" appearance, is thought to suggest incomplete anticoagulation, especially since this phenomenon occurs only at concentrations nearest to clotting.

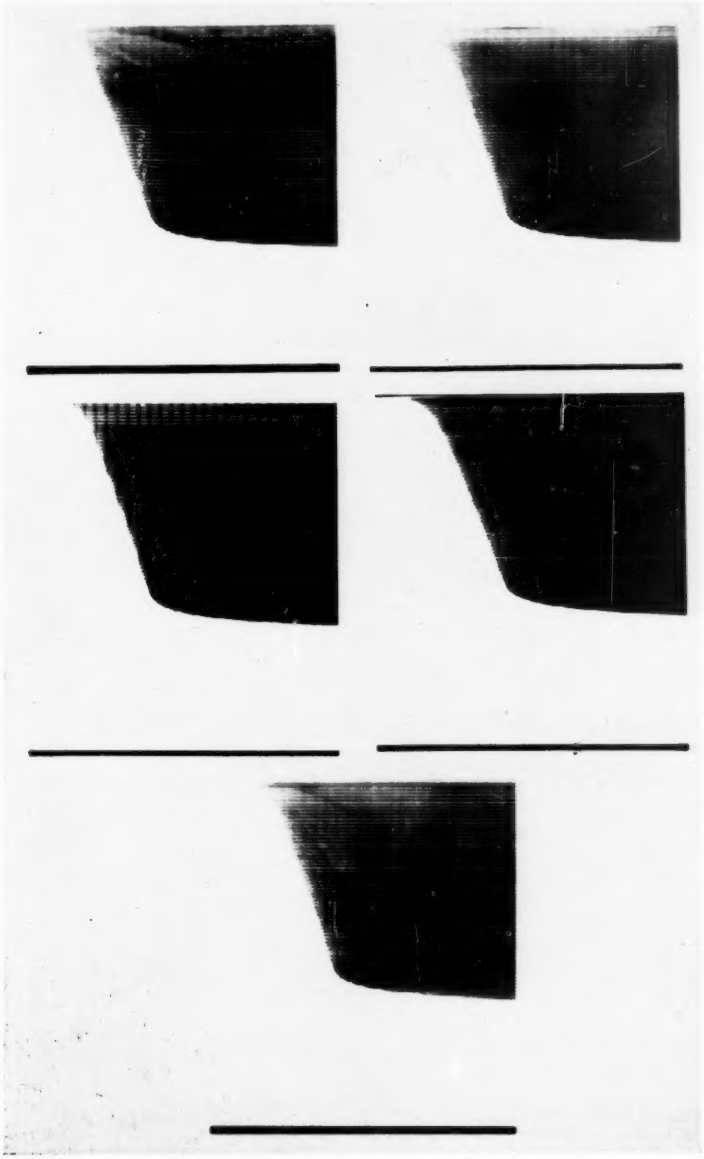

Fig. 3.—Effects of varying quantities of ammonium oxalate upon sedimentation.

In practice the ammonium and potassium oxalate mixture has been most satisfactory.

As photographic examples of the effects of those anticoagulants which decrease volume per cent as well as sedimentation consider the photographs in Fig. 2.

Anticoagulant—lithium citrate:

Ex. No.	Mg./5 c.c. blood	em	t_{em}	$\angle^{s}g$
1	8	19 mm.	16 min.	48° 20′
2	12	20 mm.	8.3 min.	31° 10′
3	16	18 mm.	8.2 min.	36°
4	20	10 mm.	7.3 min.	47° 50′
5	24	7 mm.	7.2 min.	62° 20′

Ex. No.	M_5	t_{M_5}	$\angle^{s}M_5$	V%	Plasma
1	13 mm.	15-20 min.	48° 20′	37.7	Clear
2	20 mm.	5-10 min.	37° 20′	36.5	Clear
3	19 mm.	5-10 min.	38° 40′	34.7	Clear
4	12.5 mm.	5-10 min.	49° 40′	34	Clear
5	7 mm.	5-10 min.	64°	33	d

As an example of those anticoagulants which increase volume percentage (Fig. 3):

Anticoagulant—ammonium oxalate:

Ex. No.	Mg./5 c.c. blood	em	t_{em}	$\angle^{s}g$
1	4	31 mm.	9 min.	20° 20′
2	8	29 mm.	10.7 min.	16° 50′
3	12	24 mm.	8.3 min.	16° 20′
4	16	29 mm.	9.3 min.	17° 10′
5	20	33 mm.	9.5 min.	18° 00′

Ex. No.	M_5	t_{M_5}	$\angle^{s}M_5$	V%	Plasma
1	34 mm.	5-10 min.	24° 00′	28.7	Clear
2	34.5 mm.	10-15 min.	23° 40′	28.7	Clear
3	37 mm.	5-10 min.	22° 10′	29.3	Clear
4	31 mm.	5-10 min.	26°	30+	Clear
5	33 mm.	5-10 min.	24° 40′	30.3	Clear

As an example of heparin (Fig. 4):

Ex. No.	Units/5 c.c. blood	em	t_{em}	$\angle^{s}g$
1	11	17.5 mm.	10.5 min.	32° 40′
2	16.5	22.5 mm.	9.2 min.	24° 30′
3	22	25.0 mm.	9.0 min.	23° 30′
4	27.5	23.0 mm.	8.8 min.	24° 10′
5	55	22.0 mm.	10.5 min.	23° 50′

Ex. No.	M_5	t_{M_5}	$\angle^{s}M_5$	V%	Plasma
1	20 mm.	10-15 min.	37° 20′	42+	f
2	25 mm.	5-10 min.	31° 20′	42+	Clear
3	27 mm.	5-10 min.	29° 20′	42.7	Clear
4	25 mm.	5-10 min.	31° 20′	42.7	Clear
5	23 mm.	5-10 min.	33° 20′	42.7	Clear

Fig. 5 represents peculiar curves where evidence of incomplete anticoagulation exists. Needless to say, measurements would be deceiving.

To continue, *apparent minimal quantities* of the various anticoagulants were compared on the same sample of blood with results similar to the following:

Sample No. 1:

Aliquot No.	Anticoagulants	Mg./5 c.c. blood	M_5	t_{M_5}	$\angle^{s}M_5$	V%
1	Ammonium and potassium oxalate	10 mg.	18 mm.	20-25 min.	40° 10′	40
2	Potassium oxalate	8 mg.	19 mm.	10-15 min.	39° 50′	35.7
3	Ammonium oxalate	8 mg.	18 mm.	15-20 min.	40° 10′	42.3
4	Heparin	15 units	21 mm.	10-15 min.	36°	38.7

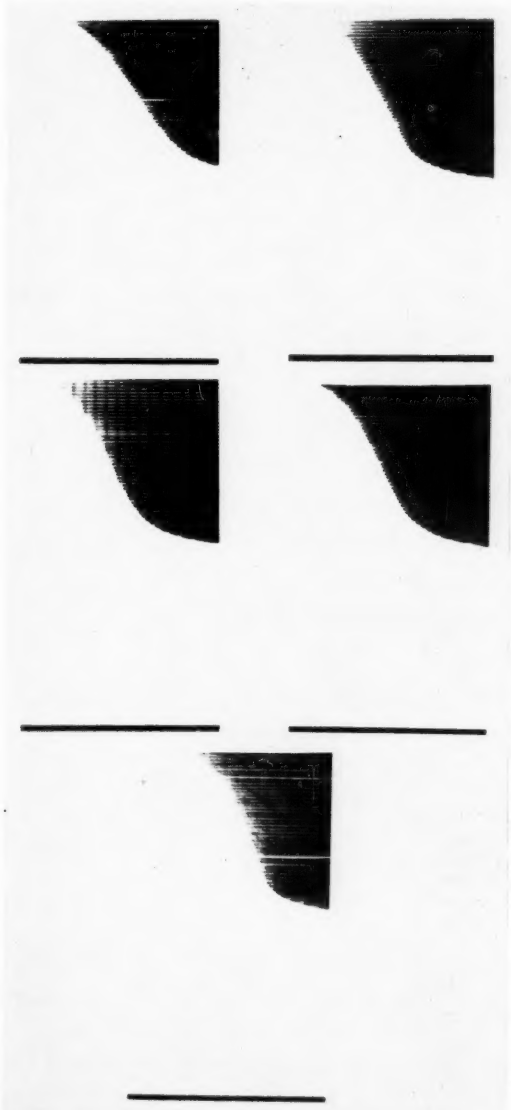

Fig. 4.—Effects of varying quantities of heparin upon sedimentation.

Sample No. 2:

Aliquot No.	Anticoagulants	Mg./5 c.c. blood	M_5	t_{M_5}	$\angle^s M_5$	V%
1	Ammonium and potassium oxalate	10 mg.	15 mm.	15-20 min.	45°	42.5
2	Lithium oxalate	8 mg.	15 mm.	15-20 min.	45°	39
3	Lithium citrate	12 mg.	15 mm.	15-20 min.	45°	39.7
4	Heparin	15 units	15 mm.	20-25 min.	45°	42.3

Sample No. 3:

Aliquot No.	Anticoagulants	Mg./5 c.c. blood	M_5	t_{M_5}	$\angle^s M_5$	V%
1	Ammonium and potassium oxalate	10 mg.	21 mm.	15-20 min.	36°	38.5
2	Potassium oxalate	16 mg.	23 mm.	10-15 min.	33° 40′	35.5
3	Sodium fluoride	12 mg.	2 mm.	10-15 min.	82° 10′	32.5
4	Heparin	15 units	24.5 mm.	10-15 min.	31° 50′	38.5

Sample No. 4:

Aliquot No.	Anticoagulants	Mg./5 c.c. blood	M_5	t_{M_5}	$\angle^s M_5$	V%
1	Ammonium and potassium oxalate	10 mg.	19 mm.	20-25 min.	39° 50′	43
2	Ammonium and potassium oxalate	4 mg.	20 mm.	15-20 min.	37° 10′	42
3	Sodium oxalate	8 mg.	19 mm.	10-15 min.	39° 20′	44
4	Ammonium citrate	12 mg.	C	-	-	-

Sample No. 5:

Aliquot No.	Anticoagulants	Mg./5 c.c. blood	M_5	t_{M_5}	$\angle^s M_5$	V%
1	Ammonium and potassium oxalate	10 mg.	20.5 mm.	15-20 min.	36° 40′	41.5
2	Sodium citrate	12 mg.	C	-	-	-
3	Sodium citrate	16 mg.	20 mm.	15-20 min.	37° 10′	43.3
4	Ammonium citrate	15 mg.	15.5 mm.	15-20 min.	44° 10′	46 -

Sample No. 6:

Aliquot No.	Anticoagulants	Mg./5 c.c. blood	M_5	t_{M_5}	$\angle^s M_5$	V%
1	Ammonium and potassium oxalate	10 mg.	17 mm.	25-30 min.	41° 40′	45 +
2	Ammonium and potassium oxalate	4 mg.	17.5 mm.	15-20 min.	41°	45 -
3	Ammonium and potassium oxalate	2 mg.	C	-	-	-
4	Sodium oxalate	8 mg.	18 mm.	15-20 min.	40° 10′	41.5

The amount of heparin used was found by previous trial to be sufficient for each particular experiment, at least as far as general appearance was concerned. Wherever any signs of clotting were apparent, or even strongly suspected, the amount of the particular anticoagulant used was increased, and the experiment was repeated. In practice it was found that even with blood from one and the same animal variations in the minimal quantity of anticoagulant occurred from time to time—this being true in both pathologic and apparently normal samples, even though perhaps more true in the pathologic blood. Realizing, of course, that large errors do occur in attempting to measure as small quantities of anticoagulants as were here used, and realizing that measuring exact small quantities of blood is also subject to large error, nevertheless in spite of these I feel that minimal quantities vary in the same animal, normal or not.

As an interesting adjunct to this problem various dilutions of these various anticoagulants were prepared and the *resistance of red blood cells* was tested in them. Results as listed are quite obvious (*h* signifies complete hemolysis; *p*, partial hemolysis; *s*, suspicious; and *o*, no hemolysis).

	Immediately	1 hour
Potassium oxalate (%):		
0.2	h	h
0.4	h	h
0.6	h	h
0.8	p	p
1.0	o	o
1.2	o	o
Sodium oxalate (%):		
0.2	h	h
0.4	h	h
0.6	s	p
0.8	o	o
1.0	o	o
1.2	o	o
Lithium oxalate (%):		
0.2	h	h
0.4	h	h
0.6	o	o
0.8	o	o
1.0	o	o
1.2	o	o
Ammonium oxalate (%):		
0.4	h	h
0.6	h	h
0.8	h	h
1.0	h	h
2.0	h	h
Half saturated (about 4%)	p	h
Saturated (about 8%)	s	h
Potassium citrate (%):		
0.2	h	h
0.4	h	h
0.6	h	h
0.8	h	h
1.0	s	p
1.2	o	o
1.4	o	o
Sodium citrate (%):		
0.2	h	h
0.4	h	h
0.6	h	h
0.8	h	h
1.0	p	h
1.2	o	p
1.4	o	o
Lithium citrate (%):		
0.2	h	h
0.4	h	h
0.6	h	h
0.8	h	h
1.0	s	h
1.2	o	o
Sodium fluoride (%):		
0.2	h	h
0.4	s	p
0.6	o	o
0.8	o	o
1.0	o	o

Ammonium citrate (%):	Immediately	1 hour	1½ hours
0.2	h	h	h
0.4	p	p	p
0.6	s	s	p
0.8	o	s	p
1.0	o	o	p
1.2	o	o	p
1.4	o	o	o
1.6	o	o	o
1.8	o	o	o
2.0	o	o	o
5.0	o	o	o
10.0	o	o	o

Four parts of ammonium oxalate and 6 parts of potassium oxalate:

Solution (%):	Immediately	1 hour
0.2	h	h
0.4	h	h
0.6	s	h
0.8	s	h
1.0	s	h
1.2	s	p
1.4	o	p
1.6	o	o
1.8	o	o
2.0	o	o

Equal parts of ammonium and potassium oxalate:

Solution (%):	Immediately	1 hour
0.2	h	h
0.4	p	p
0.6	o	p
0.8	o	s
1.0	o	s
1.2	o	o
1.4	o	o
1.6	o	o
1.8	o	o
2.0	o	o

Six parts of ammonium oxalate and 4 parts of potassium oxalate:

Solution (%):	Immediately	1 hour
0.2	h	h
0.4	h	h
0.6	s	h
0.8	s	p
1.0	s	p
1.2	o	p
1.4	o	o
1.6	o	o
1.8	o	o
2.0	o	o

Consideration of the foregoing data is interesting in that the point of beginning hemolysis in all salts except one was in solutions of less than 2 per cent. In the oxalates, with the exception of ammonium oxalate, and in sodium fluoride beginning hemolysis was in solutions of less than 1 per cent, while in the citrates, including ammonium citrate, it began in solutions between 1 and 2 per cent. All dilutions of ammonium oxalate hemolyzed the red blood cells, although a saturated solution (approximately 8 per cent) offered some protection for a fraction of an hour.

Would it be incorrect to say that oxalates except ammonium oxalate offer greater protection against cell damage than the citrates?

The reaction of ammonium oxalate is indeed an interesting one, since in all dilutions up to saturation hemolysis occurs when 1 drop of fresh blood is added to 2 c.c. of the solution. No doubt, when used alone as an anticoagulant, the quantity necessary (about 8 to 12 mg.) for anticoagulation is so small in proportion to the amount of blood used (5 c.c.) that clinically at least evidence of hemolysis is not observed. The quantity necessary for the ammonium and potassium oxalate mixture is even less (6 mg.). Another factor must be considered, namely, the possibility that potassium oxalate may somewhat protect the red blood cells against the hemolyzing effects of ammonium oxalate. Concerning

the proportion of these two salts it may be said that a slight excess of ammonium oxalate (which according to previous observations swells the cells, as evidenced by volume percentages greater than those of heparinized samples) seems necessary to overcome the shrinkage effect of potassium oxalate, such shrinkage being evidenced by volume percentages less than those of heparinized samples.

Fig. 5.—Sedimentation curves of samples with incomplete anticoagulation.

A proportion of 6 parts of ammonium to 4 parts of potassium oxalate seems to produce volume percentages closer to those of heparinized samples than other proportions or even other single salts.

In practice, between 4 and 12 mg. of the mixture of ammonium and potassium oxalate per 5 c.c. of blood have been generally considered safe. Ten milligrams per 5 c.c. as a routine quantity so far has been satisfactory. No evidence of clot has been detected in any sample as yet; photographed curves have been smooth and not irregular, as sometimes occurs with heparin. Hemolysis has not

occurred in any normal sample, and in the few pathologic samples where it has occurred, it has also occurred with heparin, and volume percentages of replicate samples as well as curves of sedimentation have been very close.

The fourth and last publication of this series will include a discussion of the effects of variations in venous stasis, delay, temperature of sample, containers, agitation, and quantity of formed elements upon the measurement of sedimentation.

REFERENCES

1. Nichols, R. E.: A Study of the Phenomenon of Erythrocyte Sedimentation. I. A Critical Survey of Literature, J. Lab. & Clin. Med. **27**: 1317, 1942.
2. Nichols, R. E.: A Study of the Phenomenon of Erythrocyte Sedimentation. II. The Establishment of a Reliable Measurement of the Phenomenon—Its Reproducibility and Limitations, J. Lab. & Clin. Med. **27**: 1410, 1942.
3. Fahraeus, Robin: The Suspension-Stability of the Blood, Acta med. Scandinav. **55**: 1, 1921.

A SHAKING MACHINE AND CONTAINERS FOR SHAKING SPUTUMS AND OTHER BODY FLUIDS DURING THE PROCESS OF HOMOGENIZATION*

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LITTLE question can be raised that marked agitation of tuberculous sputum during homogenization breaks up tenacious matter and small caseous or pussy particles, and increases the distribution of the tubercle bacilli in the specimen, thereby making possible a more accurate count of bacilli by microscopic examination of the smear.

There are many types of agitating and shaking machines from which to choose, but from the standpoint of performance and durability, the Red Devil,† or a similar type, is the most satisfactory. The model described in this article will shake a can of paint of any size up to a gallon, and the rate of agitation is 760 three-dimensional shakes per minute.

For our work a special container had to be designed for holding more than one specimen bottle in place during agitation. Also, it had to be so constructed that the bottles or cans could be placed inside the container in a way that would allow for differences in height, size, and shape, and would keep stoppers or lids firmly in place during the shaking operation.

DESCRIPTION OF CONTAINER

Fig. 1 shows a vertical section taken on the center line through the large group container, and shows vertical sections through two of the individual containers.

Fig. 2 is a plan view looking down on the group container with the top section (*1*) removed, and shows the arrangement of the individual containers within the group container.

*From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau.
†Made by Landon P. Smith, Irvington, N. J.
Received for publication, April 15, 1942.

Fig. 3 shows a vertical section taken on the center line through an individual container.

Fig. 4 is a plan view looking down with the top section (*4*) removed, and shows the specimen container in place.

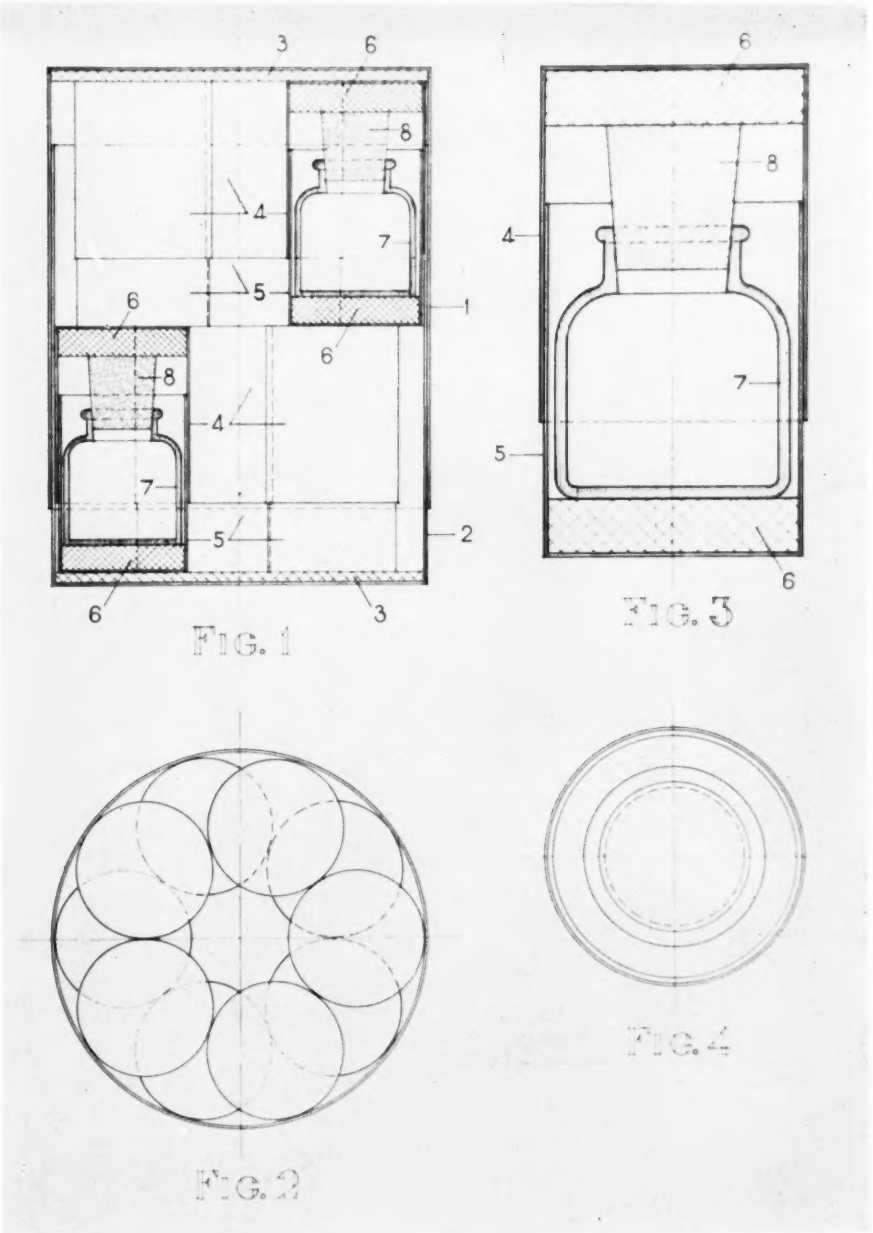

Plate 1.

The group container consists of two sections, the top section (*1*) fitting over the bottom section (*2*). It is designed to hold a given number of smaller indi-

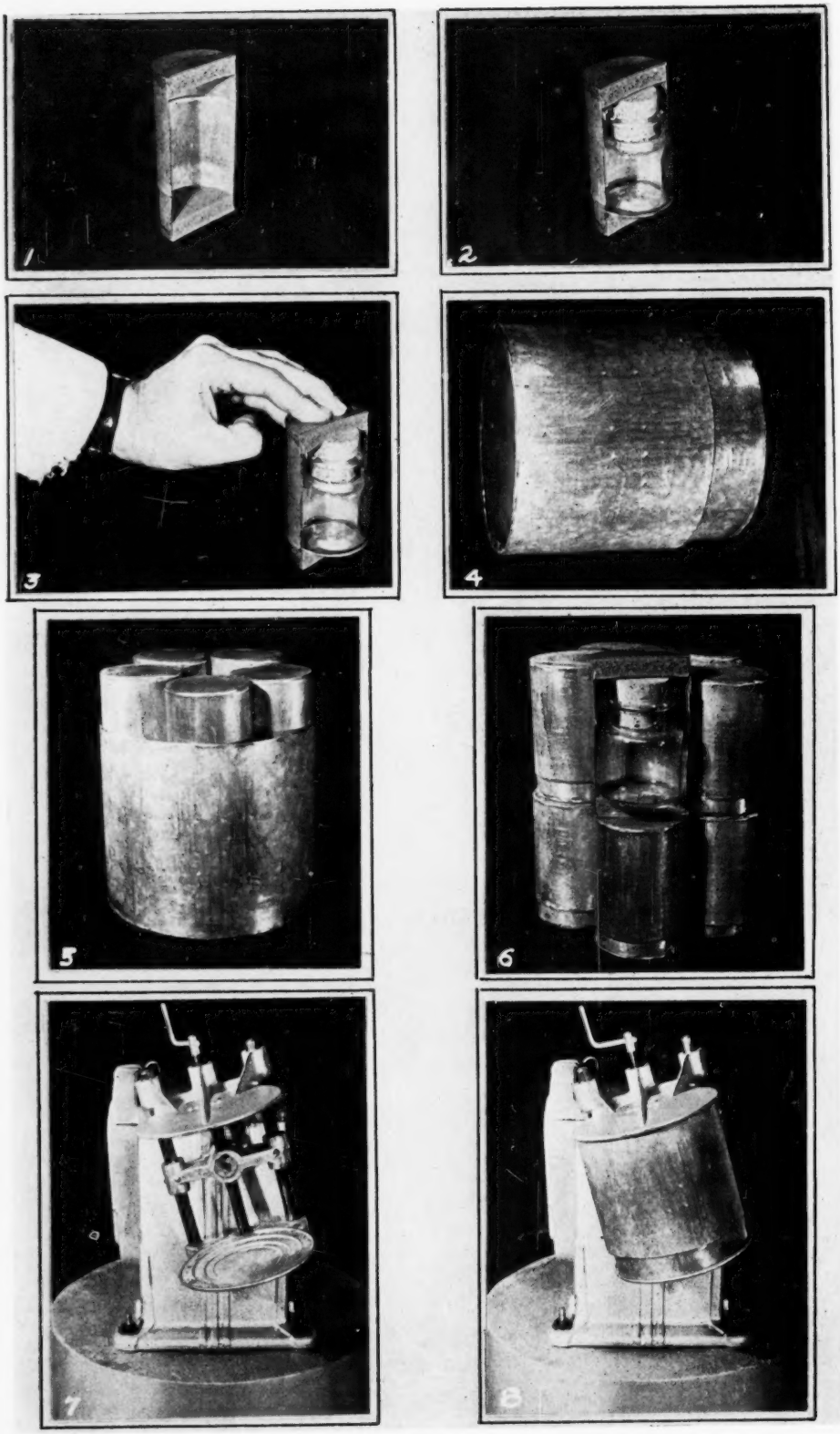

Plate 2.

vidual containers. The size of the group container is determined by the size and number of individual containers to be accommodated.

A cushion of soft rubber, or some other resilient material (*3*), is placed inside each section, as shown, to allow for variation in height of individual containers and to prevent movement of such containers during the shaking operation.

The individual container consists of two sections, the top section (*4*) fitting partly or completely over the bottom section (*5*), and it is designed to hold a specimen container (*7*). A rubber sponge cushion (*6*) is placed in each section, as shown, to compensate for the variation in height of stopper (*8*), and to provide for resiliency during the shaking process.

Plate II shows the following:

1, A cross section of an individual container.

2, A cross section of an individual container with bottle in place.

3, A cross section of an individual container with bottle in place under pressure. Note rubber compression.

4, A group container.

5, A group container with lid removed. Note also the upper layer of individual containers.

6, Shows how the individual containers stack in the group container. Also, a cross section of an individual container with bottle in place.

7, The shaking machine mounted on a concrete base which, in turn, is mounted on four rubber sponge cushions.

8, Shaking machine with group container, with individual container inside fastened in clamp and ready for shaking.

Our experience has proved that the use of this shaking machine with its specially designed containers, results in a better homogenization of sputum. The concentration of sodium hydroxide can be reduced. Those specimens that usually require 4 per cent routinely, can be digested with 3 per cent, and with as little as 2 per cent, depending upon the appearance of the sputum, thus decreasing the loss of acid fastness, and lessening the chance of killing the tubercle bacilli by the digesting mixture.

The marked agitation and speed of the shaking process make possible a more even distribution of the tubercle bacilli, and this enables us to obtain an accurate count of bacilli in concentrated smear, especially in the Gaffky I and the so-called negative specimens. By this method of shaking we have raised our positive findings 11.0 per cent, particularly in the low Gaffky counts (I, II, and III).

A SIMPLE SURGICAL TECHNIQUE FOR DEVIATING THE ENTIRE URINE INTO THE BLOOD STREAM*

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IN CONNECTION with many problems of renal physiology it is of great importance to establish whether a change elicited by damage to renal tissue is caused by interference with the excretory functions of the kidney or whether it is due to a derangement of its endocrine and metabolic activities. In such instances the technique usually employed is to compare the results of complete nephrectomy with those caused by ligature of both ureters. It is then assumed that while nephrectomy eliminates all functions of the kidney, ligature of the ureters abolishes only renal excretion. It has often been emphasized, however, that after ligature of the ureters some urine is still produced so that hydronephrosis results. Hence this technique does not eliminate excretion completely. Furthermore, the often very high pressure which develops in the renal pelvis is transmitted to the kidney cells and damages them severely, so that not only the excretory, but also all the renal functions must suffer to some extent. It should also be borne in mind that a kidney, whose excretory function is gradually abolished by back pressure, is not normal, and that inhibition of excretory work may in itself interfere with other activities of the kidney cells.

The object of this study is to describe an experimental technique which makes it possible to eliminate the result of renal excretion completely, without actually interfering with the process of urine production or with any other kidney function. The fundamental principle of this method is to prevent urine elimination by ligature of the urethra, and then to establish a communication between the bladder and the vena cava. Thus the urine which continues to be formed and accumulates in the bladder is led back into the blood. The technique is readily adaptable to most laboratory animals, but up to the present it has mainly been used for experiments on the rat.

The procedure is as follows: The rat is anesthetized with ether and tied on its back to an operating board. An incision is made through the skin and muscle layers in the midline from the pubic region to the umbilicus. The intestines are pushed craniad and the rectum is pushed to the left by means of cotton pads which can be held in position by a retractor whose other end is hooked onto some heavy object on the table. This arrangement makes it possible to obtain perfect exposure of the vena cava between the pelvis and the kidney region without the help of an assistant. Now the peritoneal covering of the posterior abdominal wall is severed by mere traction between two fingers, and the vena cava

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Received for publication, April 15, 1942.

is carefully separated from its surroundings between its bifurcation into the two iliac veins and the right iliolumbar vein. This is accomplished by blunt dissection using two curved forceps, each of which holds a small cotton pad. With the help of these pads it is easy to crush and sever the numerous little venules which enter the cava from the psoas major and the ventral surface of the lumbar vertebrae. Some of these latter venules are very large, yet they can all be severed without ligature if they are first crushed with the pads. If they tend to bleed, the hemorrhage is readily stopped by pressing a minute piece of cotton on them until clotting occurs. After this the vena cava is completely occluded by a ligature placed above the junction of the iliac veins. Another ligature is laid loosely around the cava just underneath the iliolumbar vein. It should be emphasized here that in the rat ligature of the vena cava caudad from the renal veins produces no symptoms as there is an adequate collateral circulation.

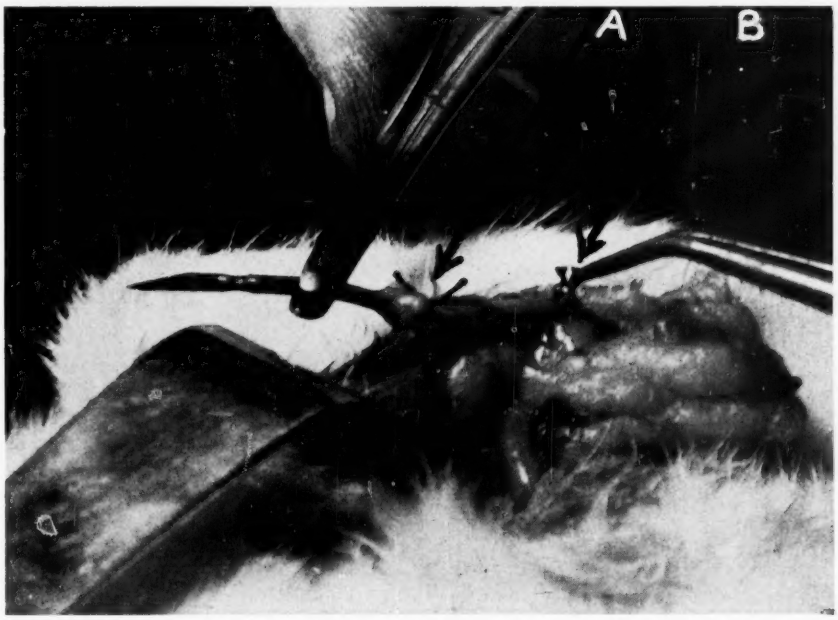

Fig. 1.—First stage of the operation. The bladder is exposed and the blunt end of the curved cannula is tied into its cranial end with the ligature shown by arrow *A*. This ligature fits into one groove of the cannula, while the other groove is visible between the pointed end and the anatomical forceps which holds the cannula. Arrow *B* points to the ligature occluding the urethra. A curved forceps holds this ligature.

Now the urinary bladder is pulled forward and a ligature is tied around the urethra just caudad from the point where the ureters enter the bladder. In order to maintain a good blood supply to the bladder it is well to avoid any interference with the large veins which accompany the ureters. After loss of urine has thus been prevented by occluding the urethra, a small incision is made on the apex of the bladder, and the blunt end of a steel cannula is introduced into the lumen. This cannula may be fastened in this position by a single ligature, as shown in Fig. 1.

The cannula, which will connect the bladder with the vena cava, is made from gauge 16 or gauge 13 injection needles by cutting off about 1 to 1½ inches

near the pointed end, making one circular groove near the blunt and one near the pointed end to hold ligatures in place. It is well to bend the cannula slightly, as shown in Figs. 1 and 2, so as to adapt them better to the anatomy of the pelvic region. In order to prevent clotting it is advisable always to use the widest gauge that can be introduced into the vena cava. If this precaution is observed, and the inner surface of the needle is heparinized, thrombi do not form, and the passage of urine into the vein proceeds readily throughout the experiment. Reflux of blood from the vein into the bladder is rarely seen, since the pressure is usually greater in the bladder than it is in the vein. Both in order to avoid reflux and as a precaution against a delay in the re-entrance of urine into the blood, most of the bladder wall should be tied off with the ligature which fastens the cannula into the bladder. Only a small stump with the ureteral orifices needs to be left intact.

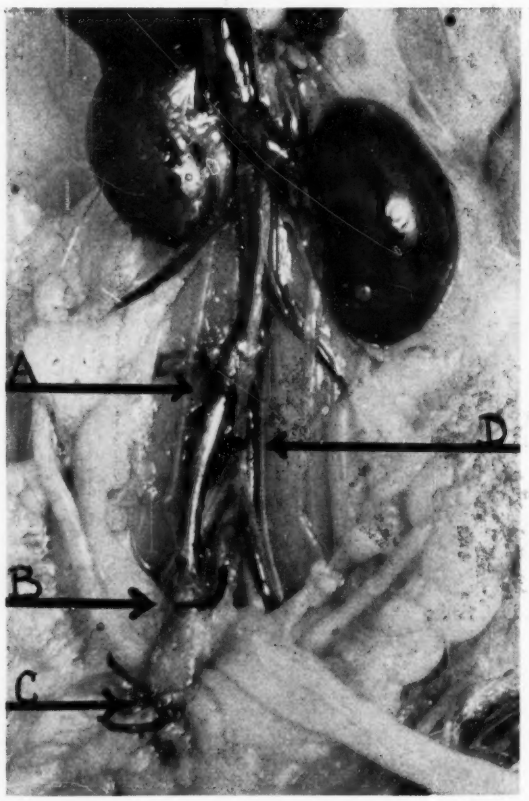

Fig. 2.—Dissection of the lower abdomen showing the position of the cannula after completion of the second stage of the operation. Arrow *A* designates the ligature which ties the vena cava to the pointed end of the cannula which is now inserted into the lumen of the vein. Arrow *B* points to the ligature fastening the blunt end of the cannula into the cranial end of the bladder. Arrow *C* points to the ligature occluding the urethra. Arrow *D* indicates the ligature occluding the vena cava above its bifurcation into the iliac veins.

The bladder is now washed with heparin which is introduced into it from a syringe armed with a fine injection needle that can readily be introduced into the free, pointed end of the cannula. It is important to aspirate into this syringe any air bubbles that may have entered into the bladder or the cannula in order

to prevent air embolisms. It is well to wipe off any excess heparin that may ooze out through the cannula, since a few drops of it suffice to prevent clotting, and an excess may lead to diffuse bleeding. Now follows the most dangerous part of the operation: The introduction of the pointed end of the cannula into the vena cava craniad from the place where the vessel was occluded and caudad from the loosely laid ligature underneath the iliolumbar vein. This is accomplished by firmly grasping the middle of the cannula with one forceps, and the vena cava with another, and then carefully pushing the point into the vessel until the circular groove underneath the pointed end is at the level of the loosely laid ligature. At this moment a few drops of blood usually escape from the vein, but bleeding is stopped immediately by pulling the ends of the loose ligature and thus fastening the wall of the cava to the cannula. A second knot fixes this ligature and thus a permanent anastomosis between urinary bladder and vena cava has been established. After removing the retractor and the cotton pads, which kept the intestines out of the way, the muscle and skin wounds are closed and the operation is finished. The intervention takes approximately fifteen minutes, and in the last series of fifty operations on rats, ranging between 105 and 275 Gm. of body weight, there was not a single case of death during the operation. Female animals are preferable because the uterus does not interfere with the cannula as much as the prostate and seminal vesicles, yet both sexes can be used.

After the animals recover from the anesthesia, they appear to be perfectly normal and readily take nourishment, showing no detectable signs of damage. Approximately twenty-four hours are required before the animals begin to show some tremor characteristic of uremia. The average survival time is thirty to forty-five hours.

Complete nephrectomy or ligature of the ureter is generally tolerated for a slightly longer time than the urinary-venous anastomosis. It is remarkable, however, that the continuous influx of urine into the blood is not accompanied by more rapidly developing signs of intoxication. It will be recalled that numerous investigators found urine to be rich in highly toxic substances, and workers who made a unilateral anastomosis between a ureter and a vein in the dog[1-4] concluded that introduction of the urine produced by one kidney suffices to elicit a rapidly developing fatal intoxication even if urine elimination through the other kidney proceeds normally. On the other hand, Geer and Dragstedt,[5, 6] who used a similar surgical procedure, observed no toxic symptoms and no rise in blood nonprotein nitrogen after unilateral anastomosis of a ureter and a vein in the dog. In certain cases their animals survived several weeks and were still in good condition at the time they were sacrificed. From this it would appear that urine in itself is not a highly toxic product.

The only investigator who attempted to connect both ureters with veins was de Almeida[7, 8] who, like all previous investigators, used the dog as an experimental animal. He employed a rather complicated method which involved the insertion of long tubes into the ureters, the tubes being led out of the peritoneal cavity and then introduced into the femoral vein. The procedure, which was successful only in a limited number of cases, led to the usual symptoms of

uremia and was fatal in a shorter period than complete nephrectomy. These experiments were also performed merely with the intent to study the toxicity of urine and, to our knowledge, the technique was never employed as a method for the selective differentiation between the excretory functions of the kidney on the one hand, and its endocrine and metabolic activities on the other hand.

The most striking pathologic changes, which we noted in our rats bearing a urinary-venous anastomosis, were a pronounced enlargement of the adrenal cortex, occasional lung edema, and a rise in the blood nonprotein nitrogen which was usually more pronounced than at a comparable period after complete nephrectomy. The marked decrease in blood hemoglobin, seen in the anastomosis-bearing animals, indicated, furthermore, that, owing to the continuous reflux of urine, blood dilution is more pronounced than after nephrectomy.

It is hoped that the use of this simple experimental technique, applicable even to small laboratory rodents, will prove useful in the study of the purely endocrine and metabolic functions of the kidney.

SUMMARY

A technique is described which permits the deviation of the entire urine into the inferior vena cava after ligature of the urethra by means of a cannula that connects the bladder with the vein. This technique permits the study of the endocrine and metabolic activities of renal tissue apart from its excretory function.

The expenses of this investigation were defrayed through a fund donated by the Des-Bergers-Bismol Co., Montreal, Canada.

REFERENCES

1. Brücke, E. T.: Ueber Versuche den Harn einer Niere dauernd in das Blut zu leiten, Wien. klin. Wchnschr. **39**: 1058, 1926.
2. Galehr, Otto, and Ito, Tadashi: Ein experimenteller Nachweis der Bildung giftiger Harnsubstanzen in der Niere, Ztschr. f. d. ges. exper. Med. **55**: 115, 1927.
3. Enderlen, E., Zukschwerdt, and Feucht: Ueber die Foldgen der Einleitung des Harnes in die Blutbahn, München. med. Wchnschr. **75**: 30, 1928.
4. Hartwich, Adolf, and Hessel, Georg: Experimentelle Untersuchungen zur Frage der Harnvergiftung, Ztschr. f. d. ges. exper. Med. **59**: 633, 1928.
5. Geer, William Allyn, and Dragstedt, Lester R.: Studies on Renal Hypertension. The Effect of Deviating Urine Into the Blood Stream and Intestines of Dogs, Ann. Surg. **108**: 263, 1938.
6. Geer, William Allyn, and Dragstedt, Lester R.: Effect of Deviating Urine Into the Blood Stream and Intestine of Dogs, Proc. Soc. Exper. Biol. & Med. **37**: 673, 1938.
7. de Almeida, A. Ozorio: L'anastomose uretéro-veineuse, nouvelle technique pour l'étude de certaines fonctions du rein, Compt. rend. Soc. de biol. **96**: 383, 1927.
8. de Almeida, A. Ozorio: Survie, débit urinaire et pression d'excrétion de l'urine chez le chien porteur d'une anastomose uretéro-veineuse, Compt. rend. Soc. de biol. **96**: 385, 1927.

THE USE OF A DETERGENT IN SOLUBILITY TESTS FOR THE IDENTIFICATION OF PNEUMOCOCCI*

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THEORETICAL considerations suggested that synthetic surface-active agents might affect pneumococci in a manner similar to that of bile and its salts. It is well known that lysis of pneumococci is activated by any process that destroys viability without impairing autolytic enzyme-activity, while it is inhibited by conditions unfavorable to such activity.[1-3] Agents that will induce the dissolution of pneumococci are legion. The principal ones, as listed by White,[4] are bile acids and salts, dilute sodium hydroxide, unsaturated fatty acids, soaps, and saponin. The most satisfactory among these is the bile salt, sodium desoxycholate, which brings about the dissolution of pneumococci at room temperature in a very few minutes.

Most of the agents mentioned depress the surface tension of the solution in which they are dissolved. The recently developed surface-active agents, some of which are long-chain alcohol sulfates, are strong surface-tension depressants. Cowles[5] showed that "duponols," in a concentration of only 0.03 per cent, prevent the growth of gram-positive bacteria. One such compound, duponol WA (flakes),† was employed by us in solubility tests.

EXPERIMENTAL WORK

A 0.2 per cent solution of duponol in distilled water was found to have exactly the same activity as a 1 per cent solution of sodium desoxycholate. When higher and lower concentrations were employed, the clearing of the solution was slower. A 0.2 per cent solution is opalescent, but when it is added to broth cultures, this property disappears. Sterilization before use is unnecessary. The solution has not been found to deteriorate, even if stored for several months. The microorganisms were cultivated in an infusion broth that contained 2 per cent peptone, but no serum, and only 0.02 per cent added dextrose. The addition of dextrose to the medium may be omitted. The dissolution of pneumococci in eighteen-hour-broth cultures was observed grossly and also by examination of films stained by Gram's method and prepared immediately after the addition of 0.1 ml. of duponol or sodium desoxycholate to 0.6 ml. of culture. Films were also prepared at the end of five minutes and of one hour.

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Received for publication, April 21, 1942.

Presented at the meeting of the New York State Association of Public Health Laboratories, Albany, November 1, 1940.

†A detergent manufactured by DuPont.

Twelve strains of pneumococci, comprising seven different types, dissolved completely in less than five minutes at room temperature under the influence of either duponol or sodium desoxycholate. All were insoluble when previously heated to 75° C. for twenty minutes. One type-specific type 14 strain and two degraded strains that had lost type specificity dissolved incompletely in both reagents, while two other strains, supposedly representing completely degraded pneumococci, appeared to be insoluble in both. Eleven strains of streptococci producing greenish discoloration of blood agar, and one strain each of hemolytic streptococcus, nonhemolytic streptococcus, and Friedländer's bacillus were insoluble in both reagents.

The pH of an eighteen-hour broth culture of pneumococcus is about 7.0 or 7.2. It is not influenced appreciably by adding the bile salt. It is lowered slightly when duponol is added, but remains well within the range of optimal enzymatic activity. The surface tension of the broth used was found to be 47.1 dynes per centimeter. The addition of sodium desoxycholate lowers the tension to approximately 44; and of duponol, to approximately 36.

Duponol was used in parallel tests with sodium desoxycholate during a four-month period in the routine examination of specimens received for pneumococcus typing. Its activity was found to compare very favorably with that of the bile salt. Broth cultures of fishings of colonies surrounded by a zone of greenish discoloration were tested; eighty-five individual strains were soluble in both reagents, two were partially soluble in both, and 78 were insoluble in both. In no instance was a discrepancy observed.

The low cost of duponol WA (flakes) compared with that of sodium desoxycholate is its principal advantage. The method has now been adopted as an alternative standard procedure in solubility tests for pneumococci in this laboratory.

SUMMARY

A detergent, duponol WA (flakes), in 0.2 per cent aqueous solution, has been found to have the same activity as 1.0 per cent sodium desoxycholate in solubility tests for differentiating pneumococci from other microorganisms producing greenish discoloration on blood agar. It has the advantage of being very inexpensive.

REFERENCES

1. Lord, F. T., and Nye, R. N.: Studies on the Pneumococcus. IV. Effect of Bile at Varying Hydrogen Ion Concentrations on Dissolution of Pneumococci, J. Exper. Med. **35**: 703, 1922.
2. Goebel, W. F., and Avery, O. T.: A Study of Pneumococcus Autolysis, J. Exper. Med. **49**: 267, 1929.
3. Dubos, R. J.: Mechanism of the Lysis of Pneumococci by Freezing and Thawing, Bile, and Other Agents, J. Exper. Med. **66**: 101, 1937.
4. White, B.: The Biology of Pneumococcus, New York, 1938, The Commonwealth Fund, pp. 55-62.
5. Cowles, P. B.: Alkyl Sulfates: Their Selective Bacteriostatic Action. Yale J. Biol. & Med. **11**: 33, 1938 (Cited by: Anson, M. L.: The Denaturation of Proteins by Synthetic Detergents and Bile Salts, J. Gen. Physiol. **23**: 243, 1939-1940.

CHEMICAL

THE NORMAL VALUE FOR THE HIPPURIC ACID LIVER FUNCTION TEST*

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QUICK[1] first described the hippuric acid liver function test in 1933. He stated that under the conditions of the test the normal excretion of benzoic acid as hippuric acid is 3 to 3.5 Gm. In 1936 he[2] suggested that the efficiency of the liver be expressed as a percentage, using 3 Gm. of excreted benzoic acid as normal. On this basis he gave the normal range as 85 to 110 per cent. In 1940 he[3] improved the original method by the addition of 5 Gm. of ammonium sulfate for each 10 c.c. of urine to decrease the solubility of the hippuric acid. At this time he gave 90 to 120 per cent as normal excretion, and suggested that an excessive excretion, i.e., over 120 per cent, might be abnormal.

After we started using the ammonium sulfate method routinely, we noticed that many of our values were over 120 per cent. Some were as high as 150 per cent. Several patients who had previously been tested by the old method gave consistently higher results with the new method. In some cases the variation between the two methods amounted to 20 per cent. Theoretically, the difference between the additive corrective factors (0.33 Gm. in the old method and 0.1 Gm. in the new), used for the amount of hippuric acid remaining in solution in the urine, should make the results by the two methods essentially identical. Since this was not found to be the case, it seemed desirable to investigate the normal excretion of hippuric acid and the factors that may influence it.

PROCEDURE

Hippuric acid tests were run on 30 men and 25 women, all presumably normal, and between the ages of 20 and 30 years, the majority being between 20 and 25 years of age. The subjects were either students or laboratory technicians. Six grams of sodium benzoate were dissolved in 30 c.c. of water and taken after breakfast. This was followed by one-half glass of water. The bladder was emptied immediately before the test, and hourly urine specimens were collected for four hours. Each specimen was measured and, if over 100 c.c., it was acidified with a few drops of acetic acid and concentrated over a water bath to about 50 c.c. The four specimens were pooled, as recommended

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Received for publication, June 10, 1942.

by Snell and Plunkett.[4] The pooled specimen was measured and divided in equal parts. To one portion 5 Gm. of solid ammonium sulfate were added for each 10 c.c. of urine. When the salt was dissolved, the urine was filtered and then acidified with concentrated hydrochloric acid until the urine was distinctly red to Congo red paper. After vigorous stirring, the specimen was allowed to stand for thirty minutes in the icebox before separating the crystalline hippuric acid by filtration through a Buchner filter. The crystals were washed with ice cold water, then dissolved in hot water. The amount of hippuric acid was determined by titrating with 0.2 N. sodium hydroxide, using phenolphthalein as an indicator. One cubic centimeter of 0.2 N. sodium hydroxide equals 0.0358 Gm. of hippuric acid. This method checked very well with the gravimetric method. We had adopted the titration method, since most clinicians in hospitals like their reports the same day the test is performed; and with the gravimetric method it was necessary to let the hippuric acid dry overnight in order to obtain uniform results. The aliquot portion was treated in a similar manner except that no ammonium sulfate was added. The calculation for the two methods is identical except for the addition of the amount of hippuric acid remaining in solution in the urine. With the method using ammonium sulfate, 0.1 Gm. of hippuric acid is added for each 100 c.c. of urine, and 0.33 Gm. per 100 c.c. is added in the method not using ammonium sulfate. These are the amounts assumed by Quick to remain in solution. The amount of hippuric acid was multiplied by 0.68 to change it to benzoic acid.

DISCUSSION

Not all the hippuric acid excreted in the urine is precipitated by the methods used. Quick, therefore, corrected his results by adding 0.33 Gm. per 100 c.c. of urine to the value found without the addition of ammonium sulfate, and 0.1 Gm. to the value obtained when the sulfate was added. The results with each method are stated as a percentage, obtained by dividing the "corrected" amount of benzoic acid excreted as hippuric acid by the arbitrarily chosen normal excretion of 3 Gm., and multiplying by 100.

The estimated amount of benzoic acid excreted as hippuric acid in the group of women ranged from 75 to 149 per cent with the old method, that is, the method not using ammonium sulfate. In the group of men the amount ranged from 110 to 156 per cent. With the new method, using ammonium sulfate, the range of the estimated amount for women was 95 to 155 per cent and, for men, 114 to 166 per cent. The difference between the two methods for each person varied greatly. The greatest increase of the ammonium sulfate method over the old method was 33 per cent. In four cases results by the old method were greater than those obtained by the use of ammonium sulfate. However, the uncorrected value in each case was greater by the latter method, the difference being due to the larger quantity (0.33 Gm.) added to "correct" the value by the method not using ammonium sulfate. The variation in the two methods can be explained on the basis of the variability in the precipitation of the hippuric acid in the urines without the addition of ammonium sulfate. The addition of ammonium sulfate to the urine gives a more con-

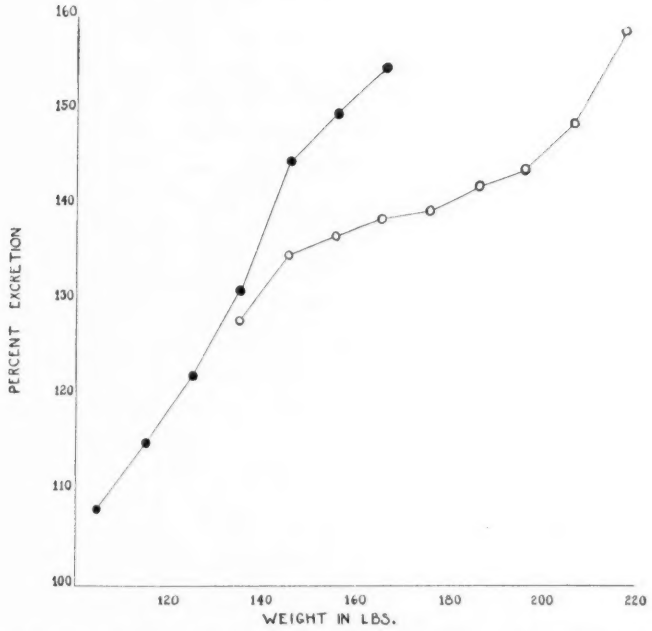

Fig. 1.—Per cent of excretion of hippuric acid as benzoic acid in relation to weight.
●—● Women.
○—○ Men.

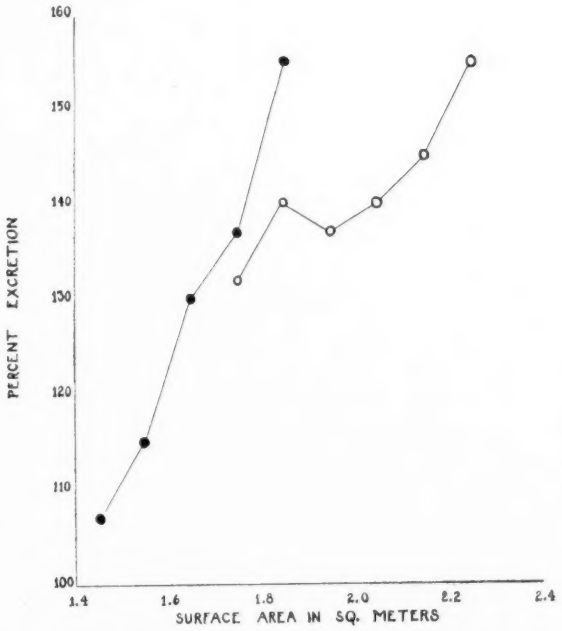

Fig. 2.—Per cent of hippuric acid as benzoic acid in relation to surface area.
●—● Women.
○—○ Men.

sistent and accurate estimation of the hippuric acid. Weichselbaum and Probstein[5] demonstrated that, with Quick's original method, the amount of hippuric acid that remained in solution was greater than 0.33 Gm. per 100 c.c. and showed a large range of variability with different urine specimens. They suggested using sodium chloride to decrease the solubility of hippuric acid. However, Marron[6] found that ammonium sulfate was the best salt to use for reducing the solubility of hippuric acid.

The statistical analysis of the data obtained in these tests is shown in Table I. From this table it is seen that the mean body weight, the mean surface area, and the mean per cent excretion is much greater in men than in women. The coefficients of correlation between body weight and per cent excretion (+0.873) and between surface area and per cent excretion (+0.834) in the women of this group was much higher than in the men (+0.545 and +0.429, respectively). The coefficient of correlation between body weight and per cent excretion in the men, in the women, and in the combined group was higher than that between surface area and per cent excretion in the same groups.

TABLE I

STATISTICAL ANALYSIS OF DATA

	MEN	WOMEN	MEN AND WOMEN
Body weight and per cent excretion*			
Body weight			
Mean	172.60 ± 2.57	125.80 ± 2.12	152.60 ± 2.71
Standard deviation	21.00 ± 1.84	16.48 ± 1.57	29.74 ± 1.91
Per cent excretion			
Mean	140.86 ± 1.60	124.60 ± 2.04	137.60 ± 1.47
Standard deviation	12.44 ± 1.08	15.86 ± 1.51	16.10 ± 1.09
Correlation coefficient	+.545 ± 0.095	+.873 ± 0.032	+.677 ± 0.049
Surface area and per cent excretion*			
Surface area			
Mean	1.99 ± 0.02	1.62 ± 0.016	1.892 ± 0.022
Standard deviation	0.147 ± 0.02	0.12 ± 0.011	0.229 ± 0.015
Per cent excretion			
Mean	140.86 ± 1.60	124.80 ± 2.12	137.60 ± 1.47
Standard deviation	12.44 ± 1.08	16.48 ± 1.57	16.10 ± 1.09
Correlation coefficient	+.429 ± 0.110	+.834 ± 0.041	+.613 ± 0.058

*Per cent excretion $= \frac{\text{Number of grams excreted as benzoic acid}}{3 \text{ Gm.}} \times 100.$

The relation of excretion to body weight is also shown in Fig. 1. These curves were made from weighted averages for each 10 pounds of weight for the men and women separately. None of these persons were obese. The variations in weight were due to differences in general build, muscular development, and stature. In such a group the size of the organs may be assumed to vary directly with the size of the body as a whole. A person with a large well-developed body will have a larger liver. This being true, it may be assumed that the liver forms hippuric acid from benzoic acid in proportion to its size. It is not surprising, therefore, to find that the per cent of benzoic acid excreted as hippuric acid is greater in larger built persons than in smaller built ones. For this reason, the normal standard for such excretion should not be fixed within even fairly wide limits but should be related to the body weight of the patient.

It is interesting to note that the coefficient of correlation between per cent of excretion and both the body weight and the surface area was much higher in the women of this group than in the men. This is also shown in Figs. 1 and 2. The curve for the women is much steeper and straighter in each case than that for the men. This may be due in part to wider range of weights of the men (135 to 220, or 85 pounds) as compared with the range in the women (103 to 167, or 64 pounds). However, while the actual surface areas of the men were, in general, greater than in women, the spread between the extremes in each sex was almost identical—for women, 1.43 to 1.87, or 0.44 sq. m.; for men, 1.78 to 2.26, or 0.48 sq. m.

We do not agree with Rosenberg and Soskin[7] that a hypersecretion of hippuric acid is pathologic, at least in all cases. We believe that the weight of the patient must be taken into consideration. Although Quick[8] definitely stated in one of his early articles that the quantitative results indicated that the excretion of hippuric acid depended on the size of the person, that is, on the surface area, this has not been emphasized in the routine use of the test. Consequently high values have been misinterpreted.

CONCLUSIONS

1. The addition of ammonium sulfate to the urine to decrease the solubility of hippuric acid makes the hippuric acid liver function test more accurate and consistent in results.

2. The lower limit of normal when ammonium sulfate is used in the test should be 90 per cent.

3. There should be no upper limit for normal because there is a correlation between body weight and the benzoic acid excreted as hippuric acid.

REFERENCES

1. Quick, A. J.: The Synthesis of Hippuric Acid: A New Test of Liver Function, Am. J. M. Sc. **185**: 630, 1933.
2. Quick, A. J.: Clinical Value of the Test for Hippuric Acid in Cases of Diseases of the Liver, Arch. Int. Med. **57**: 544, 1936.
3. Quick, A. J.: The Clinical Application of the Hippuric Acid and the Prothrombin Tests, Am. J. Clin. Path. **10**: 222, 1940.
4. Snell, A. M., and Plunkett, J. E.: The Hippuric Acid Test for Hepatic Function; Its Relation to Other Tests in General, Am. J. Digest. Dis. & Nutrition 2: 716, 1936.
5. Weichselbaum, T. E., and Probstein, J. G.: Determination of Hippuric Acid in Urine, J. Lab. & Clin. Med. **24**: 636, 1939.
6. Marron, T. U.: Effect of Added Salts on Recovering of Hippuric Acid From Urine, J. Lab. & Clin. Med. **27**: 108, 1941.
7. Rosenberg, D. H., and Soskin, S.: Comparison of the Cephalin-Cholesterol Flocculation Test With Various Criteria of Liver Function, Am. J. Digest. Dis. **8**: 421, 1941.
8. Quick, A. J.: The Conjugation of Benzoic Acid in Man, J. Biol. Chem. **92**: 65, 1931.

DETERMINATION OF ALUMINUM IN BIOLOGICAL MATERIAL WITH THE EVELYN PHOTOELECTRIC COLORIMETER*

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ALTHOUGH numerous quantitative tests for aluminum have been described in the literature, no method has proved entirely satisfactory. A major technical problem has been the development of a stable, colored lake free of interfering substances and suitable for colorimetric analysis. In the course of a study on the effect of aluminum hydroxide and aluminum phosphate on mineral excretion in man, an improved colorimetric technique for the determination of aluminum was developed which overcomes this difficulty and permits the rapid and accurate quantitative measurement of aluminum in biological material.

PRINCIPLE OF PROCEDURE

The material to be tested for aluminum is put into solution and brought to a pH of 4.9. Aurintricarboxylic acid is added, and the mixture is heated to from 70° to 78° C. until a red color develops, the intensity of which is measured in a colorimeter.

$$Al + \text{aurin} \rightarrow \text{red color}$$
$$H_2O + \text{aurin} \rightarrow \text{yellow color}$$

Rapid and reproducible reading of samples for their aluminum content is further facilitated by a chart which converts the values obtained from the colorimeter into milligrams of aluminum. This chart may be prepared by plotting the readings of light intensity obtained from solutions of known aluminum content on semilogarithmic paper, and interpolating from the standard curve.

DEVELOPMENT OF A STANDARD CURVE

Reagents:

1. *N/10 HCl*—8.3 ml. of 37 per cent (concentrated) HCl (reagent grade) diluted to one liter with distilled water.
2. *Aluminum standard*—3.5018 Gm. of $K_2SO_4.Al_2(SO_4)_3.24H_2O$ are dissolved and diluted to one liter with N/10 HCl (stock solution). Dilute 1:20 with N/10 HCl to give a solution containing 0.01 mg. Al per milliliter.
3. *Phthalate buffer*—M/5 potassium acid phthalate ($KHC_8H_4O_4$). 20 Gm. of potassium biphthalate in 500 ml. of water.
   250 ml. of M/5 phthalate and 238 ml. of N/10 NaOH made up to one liter with water. The pH should be 4.9 to 5.1.

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Received for publication, May 18, 1942.

4. *0.1 per cent aurintricarboxylic acid*—(Eastman Kodak P 4468.)
   1 Gm. of aurin and 2.5 ml. of concentrated NH_4OH diluted to one liter with water. Keep in a brown bottle. A good quality of aurin gives a straw-yellow color in a dilution of 1:100 ml.
5. *1:50 NH_4OH*—1 ml. of concentrated (28 per cent) NH_4OH made up to 50 ml. with water.
6. *Methyl red*—0.2 per cent alcoholic solution.
7. *Ammonium thiocyanate* (or potassium thiocyanate)—60 per cent. Keep in a brown bottle.
8. *Ether*—exposed to light or containing some alcohol.

Standards:

Ten standards of varying concentrations of aluminum are prepared from the stock solution to contain from 0.005 mg. per milliliter to 0.05 mg. per milliliter.

Blank:

Since the pH affects the color of the aurin itself without the addition of aluminum, it is important to have the blank at the same pH as the standards or unknowns. Accordingly, the blank contains all the reagents except the aluminum.

PREPARATION OF A STANDARD CALIBRATION CURVE

0.42 ml. of 1:50 NH_4OH*
5.00 ml. of phthalate buffer
1.0 ml. of standard
1.0 ml. of aurin

Three such tubes are prepared for each dilution of standard in addition to three blanks. The blank contains 1 ml. of tenth-normal hydrochloric acid in place of a standard solution. After the addition of the aurin the tubes are shaken gently to mix the contents and placed in the test tube rack. When all tubes are set up and stoppered, the rack is placed in a water bath for exactly five minutes at a temperature between 70° and 78° C. The rack with the tubes is then removed from the bath and immersed in a deep pan of cold water for five minutes, or until the tubes reach room temperature. The water level in the pan should extend at least to the level of the solution inside the tubes. The solution is then diluted to the final volume of 25 ml. The first few milliliters are used to wash down any solution condensed on the walls of the tube into the main body of the liquid, and then the remainder is allowed to run freely into the middle of the tube to aid in mixing the solution. The tube is then moved in a circular motion until the color is evenly distributed throughout. Inverting the tube to mix the contents is faster, but any loss of fluid introduces an error in the reading. The standards are then ready to be read in the colorimeter. The reading of the "blank" is subtracted from that of each standard. The galvanometer readings, indicating the amount of light transmitted, are then plotted against the concentration of aluminum, using semilogarithmic paper. The values from this prepared chart can be used indefinitely to determine the concentration of aluminum in any unknown solution prepared under identical conditions.

*One milliliter of standard requires 0.42 ml. of 1:50 NH_4OH for neutralization to methyl red.

CENTER POINT

The colorimeter is adjusted to read 100 per cent light transmission with the tube containing water in the path of the light. The tube is then removed and the galvanometer reading taken as the "center point." The same "center point" is kept throughout the reading of all the unknowns prepared at the same time.

TABLE I

RECOVERY OF ALUMINUM IN THE FECES DURING ORAL ADMINISTRATION OF ALUMINUM HYDROXIDE AND ALUMINUM PHOSPHATE

ANTACID GIVEN	CASE I		CASE II	
	4-DAY INTAKE Al (MG.)	4-DAY OUTPUT Al (MG.)	4-DAY INTAKE Al (MG.)	4-DAY OUTPUT Al (MG.)
Aluminum hydroxide	4,716	4,866	4,716	4,986
Aluminum phosphate	2,625	2,787	2,625	2,697
Control		13		15

TABLE II

RECOVERY OF ALUMINUM CHLORIDE ADDED TO SAMPLES OF FECES

	ALUMINUM ADDED (MG./ML.)	ALUMINUM FOUND (MG./ML.)
2 Gm. of feces	0.0120	0.0123
2 Gm. of feces	0.0120	0.0122
2 Gm. of feces	0.0120	0.0122

Blank determinations on diluted urines, plasma, feces and distilled water, known to be free of aluminum but ashed, extracted, and treated with the reagents, gave values of 0.0002 to 0.0003 mg. per milliliter.

PREPARATION AND ANALYSIS OF UNKNOWN

Feces.—The quantity of feces used for analysis depends upon the conditions of the clinical experiment. A patient on an ordinary diet excretes a very small amount of aluminum in the stool, so that it is advisable to use 10 Gm. (wet weight) of feces for an analysis. During aluminum therapy 2 Gm. of feces are sufficient.

For *ashing*, 2 Gm. of fresh, moist, well-mixed feces are weighed into a vitreosil silica beaker and 0.2 ml. of nitric acid, and 0.2 ml. of sulfuric acid are added. The water is driven off by heating at 100° C. in a drying oven. It is then ashed in an electric muffle furnace for about twenty-four hours at a temperature of 400° to 500° C. The heat should be started slowly to avoid spattering of the material. When a fluffy white ash is obtained, 10 ml. of tenth-normal hydrochloric acid are added to the ash, which is transferred to a centrifuge tube where the insoluble material is separated by centrifuging. According to the expected concentration of aluminum, aliquots of the supernatant fluid are further diluted 25 to 50 times.

If iron is present in the solution, it can be removed after bringing it to volume by adding 60 per cent ammonium thiocyanate and ether to a portion and shaking it several times in a separatory funnel or centrifuge tube. The colored ether extract is siphoned off, and the aluminum is determined on 1 ml. aliquots of the colorless substrate, exactly as in the calibration of the standard curve.

Urine.—One hundred grams of urine are weighed into a vitreosil beaker, 0.2 ml. of concentrated sulfuric acid and 0.2 ml. of nitric acid are added, and the solution is evaporated to dryness, charred in a drying oven, and then ashed in a muffle furnace for about twenty-four hours at a temperature between 400° and 500° C. Five milliliters of tenth-normal hydrochloric acid are added to the ash. The solution is then centrifuged. Two milliliters duplicate samples of the clear supernatant liquid are placed in colorimeter tubes and evaporated to dryness in an oven at 100° C. One milliliter of tenth-normal hydrochloric acid is then added to the dried residue in the tubes, and the color is developed exactly as for feces. It is sometimes advisable to combine the ash of several samples to increase the concentration of aluminum. Another method is to add a known amount of aluminum to bring the color into a better range for reading.

The concentration of aluminum in the urine is extremely low (0.005 to 0.15 milligrams per liter) even during aluminum therapy, and there are large variations from day to day in the same person on a constant intake of aluminum.

TABLE III

RECOVERY OF ALUMINUM IN URINE DURING ORAL ADMINISTRATION OF ALUMINUM HYDROXIDE AND ALUMINUM PHOSPHATE

	CASE I		CASE II	
ANTACID GIVEN	24-HOUR INTAKE Al (MG.)	24-HOUR OUTPUT IN URINE Al (MG.)	24-HOUR INTAKE Al (MG.)	24-HOUR OUTPUT IN URINE Al (MG.)
Control		0.014		0.052
	--	0.015	--	0.023
Aluminum hydroxide	1,179	0.043	1,179	0.075
	1,179	0.125	1,179	0.091
Aluminum phosphate	656	0.041	656	0.125
	656	0.222	656	0.139

TABLE IV

DETERMINATIONS OF ALUMINUM IN THE BLOOD OF PATIENTS ON ANTACID THERAPY

CASE	CONTROL Al (MG./100 ML.)	TYPE OF THERAPY	
		ALUMINUM PHOSPHATE (MG./100 ML. Al)	ALUMINUM HYDROXIDE (MG./100 ML. Al)
I	0.020	0.040	0.045
	0.015	0.045	0.050
	0.022	0.049	0.048
II	0.050	0.053	0.070
	0.045	0.046	0.049
	0.048	0.049	0.050
III	0.030	0.040	0.045
	0.022	0.030	0.040
	0.040	0.046	0.048

Blood.—Fresh oxalated blood is mixed well and centrifuged. Ten to 15 ml. of plasma are placed in a vitreosil silica beaker, 0.2 ml. of nitric acid and 0.2 ml. of sulfuric acid are added, and the solution is dried in an oven at 100° C. The sample is then ashed in a muffle furnace for twenty-four hours at 400° C., or until the ash is white. The ash is dissolved in 5 ml. of tenth-normal hydrochloric acid and transferred to a centrifuge tube. It is then centrifuged to remove the insoluble material. Two milliliter duplicates of the clear super-

natant liquid are placed in colorimeter tubes and evaporated to dryness in an oven at 100° C. When dry, 1 ml. of tenth-normal hydrochloric acid is added and the other reagents, as for the urine and feces.

Amounts of aluminum recovered from the blood of patients excreting about 2 to 4 mg. per twenty-four hours in the feces vary from 0.0113 to 0.0984, with an average of 0.045 mg. per 100 ml.

Recovery Experiment.—A solution containing a known amount of aluminum was added to samples of plasma and ashed, as described above. The results are recorded in Table V.

TABLE V

RECOVERY OF ALUMINUM ADDED TO PLASMA

ALUMINUM IN PLASMA	ALUMINUM ADDED (MG.)	ALUMINUM RECOVERED (MG.)	ALUMINUM CALCULATED (MG.)
0.0180	0.0050	0.0230	0.0230
0.0175	0.0050	0.0224	0.0225
0.0510	0.0050	0.0554	0.0560
0.0540	0.0050	0.0591	0.0590

EXPERIMENTAL CONSIDERATIONS

1. *Influence of pH and temperature on the color of the lake formed by the aluminum-aurin complex.* Eveleth and Myers concluded,[3] after a critical review of the colorimetric analyses for aluminum currently in use, that the lake formed with aurintricarboxylic acid as a chromatogenic agent was superior to that formed with alizarin, or with 1, 2, 5, 8-hydroxyanthraquinone. On the basis of their work, aurin was used in this test. It is not an ideal reagent for colorimetric work, however, since it is not colorless and varies with the pH and temperature. Thus, in a solution of pH 7.0 or above, it is yellow; at a pH of 3 to 5 it has a faint orange-pink color. Various investigators have sought to overcome this difficulty by suggesting a final adjustment of the solution to a pH 7.2, using ammonium chloride for the purpose of removing any excess color. This procedure is difficult to control, since the lake is readily decomposed or its color progressively fades.

In the present test the pH of the aluminum solution was adjusted before the color was developed and maintained at a constant hydrogen-ion concentration by means of a buffer. The acid solution of digested biological material was made neutral to methyl red with a small amount of ammonium hydroxide. A phthalate buffer at pH 4.9 to 5.1 was used because potassium biphthalate was easier to handle than the hygroscopic ammonium acetate used by some investigators for an acetate buffer. By the foregoing procedure a solution was obtained which had a final pH of 5.1. At this pH a definite red lake formed in the presence of aluminum. The color, however, developed slowly at room temperature. When heated in a water bath at 70° to 78° C., it developed a maximum intensity in five minutes.

2. *Prevention of the precipitation of the colloidal color complex at high concentrations of aluminum.* Previous investigators reported precipitation of the lake when the concentration of aluminum exceeded 0.015 mg.[22] To obtain a curve for the photoelectric colorimeter, it was desirable to keep the lake in a

dispersed phase well beyond a concentration of 0.05 mg. The stability of the lake was not increased by changes in pH after the development of color; it was less stable when large amounts of ammonium salts were added. Attempts to peptize the precipitate by adding large amounts of water were unsuccessful. Using organic solvents in diluting the colored lake did not prevent it from precipitating. Heating above 80° C. increased the tendency of the lake to precipitate. When more than 1 ml. of aurin was used, it was less stable.

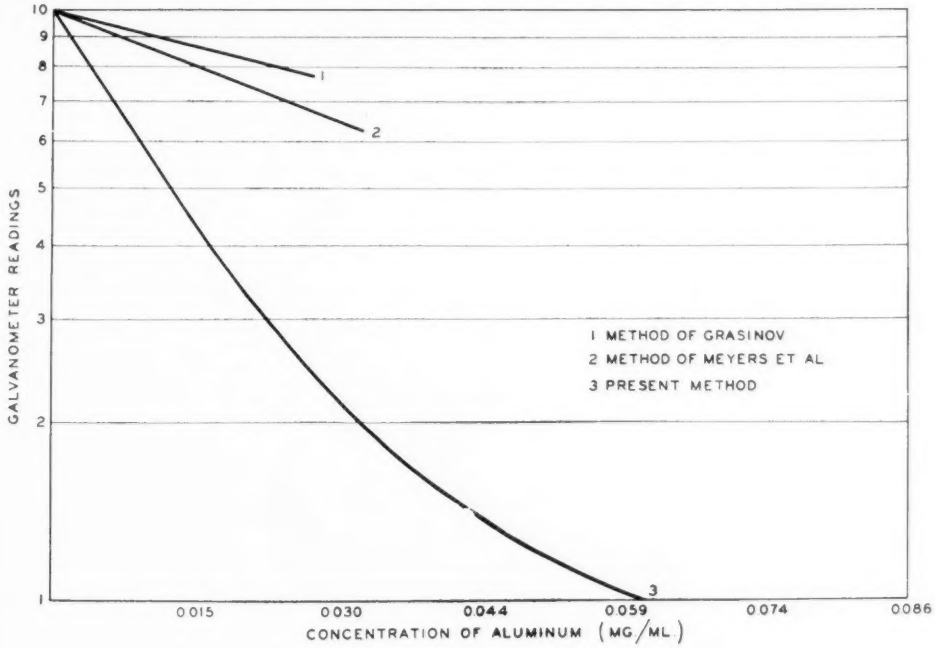

Chart 1.—Comparison of methods for sensitivity.

This problem was solved by the following procedure: The aluminum-aurin lake was allowed to form in a volume of solution not exceeding 10 ml., and was heated at 70° to 78° C. for five minutes. After cooling, it could be diluted with water to any desired volume without danger of precipitation. A solution containing as much as 0.063 mg. of aluminum in 25 ml. remained stable for an indefinite period of time (Chart 1).

3. *Optimum quantity of aurin reagent for developing a color with aluminum.* Amounts of aurin reagent, varying from 0.16 ml. to 2.0 ml., were tested. One milliliter yielded the best results. An amount of aurin less than 1 ml. was insufficient to contrast differences in concentration of aluminum; more than 1 ml. gave too much color to the blank.

4. *Elimination of interfering substances.* Yoe,[23] after investigating certain metallic ions which might form colored lakes similar to that of aluminum with aurin, found that all except iron were decolorized by ammonia.

A qualitative test to determine whether iron is present in the sample should be made before the development of color. Sixty per cent of ammonium thiocyanate, followed by an ether extraction, completely removed the interfering substance when present.

5. *The final dilution.* For the greatest accuracy in photoelectric colorimetry, the intensity of light transmitted by the colored solution should cause a deflection of the galvanometer needle within the most sensitive range of the instrument. Most of the galvanometer readings should fall, therefore, between 80 and 20 on the galvanometer scale. The final volume of 25 ml. was found, experimentally, to meet this requirement.

6. *Selection of filter.* The filter was selected from a spectrophotometric analysis of the colored solution. The characteristic peak of maximum transmission was between 520 and 540 mμ, at about 530 mμ. The Evelyn filter No. 520 was slightly more sensitive than No. 540.

COMMENT

Analyses of feces, urine, and plasma for aluminum before and after the administration of aluminum phosphate and aluminum hydroxide indicate that the aluminum is excreted entirely by way of the gut and that there is very little, if any, absorption into the blood stream. Kehoe and co-workers[7] found "the daily output in the feces . . . practically equivalent to daily intake in the diet," when speaking of the trace metals, such as aluminum, normally found in the body. In agreement with this observation the figures in Table I show a quantitative recovery of aluminum in the feces during aluminum therapy.

These same authors[7] report the concentration of aluminum in normal urine as 0.052 mg. per liter ± 0.025 mg. The values given for control urines in Table III of the present study are 0.015 mg. and 0.052 mg. Since the concentration of aluminum in the urine varies from day to day in the same person on a constant diet, sharp margins of normal are not easily established.

Grasinov[4] reported 0.068 to 0.0708 mg. of aluminum in 100 Gm. of whole blood. Kehoe[7] found 0.015 mg. ± 0.012 mg. of aluminum per 100 Gm. of whole blood "occurring almost entirely in the plasma." The values in Table IV of this present study, based on analyses of plasma, range from 0.015 to 0.050 mg. per 100 c.c.

From these comparisons it may be concluded that the results of the present method are in agreement with those of previous investigators.

CONCLUSIONS

1. An improved method for the quantitative determination of aluminum in biological material, using the Evelyn photoelectric colorimeter, is described. The use of a phthalate buffer, a smaller volume of reaction mixture, and careful control of the pH and temperature of the solution, as well as of the time of dilution, contribute to the formation of a stable-colored solution. The procedure is easily performed and yields accurate and reproducible results.

2. There is no significant increase in the aluminum content of the blood or urine after the oral administration of aluminum hydroxide or aluminum phosphate.

REFERENCES

1. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists; Washington, D. C., 1935, Association of Official Agricultural Chemists, p. 122.

2. Balanescu, G., and Matzoc, M. D.: Determination of Aluminum With 8-Hydroxquinoline in the Presence of Iron and Phosphoric Acid, Ztschr. f. anal. Chem. **118**: 18, 1940.
3. Eveleth, D. F., and Myers, V. C.: Studies on Aluminum. I. A Critical Study of Quantitative Colorimetric Methods for Aluminum on Biological Material, J. Biol. Chem. **113**: 449, 1936.
4. Grasinov, P. N.: Aluminum in Blood and Cerebrospinal Fluid, Bull. biol. et méd. exper. USSR. **7**: 88, 1939.
5. Hammett, L. P., and Sottery, C. F.: A New Reagent for Aluminum, J. Am. Chem. Soc. **47**: 142, 1925.
6. Kehoe, Robert A., Cholok, J., and Story, R.: A Spectrochemical Study of the Normal Ranges of Concentration of Certain Trace Metals in Biological Material, J. Nutrition **19**: 579, 1940.
7. Kehoe, Robert A., Cholok, J., and Story, R.: Manganese, Lead, Tin, Aluminum, Copper, and Silver in Normal Biological Material, J. Nutrition **20**: 85, 1940.
8. Korenman, I.: Flotation Reaction for Aluminum, J. Applied Chem. (USSR) **13**: 309, 1940.
9. Kul'berg, L. M., and Rovinskaya, E.: Determination of Traces of Aluminum With Aluminon, Zavodskaya Lab. **9**: 145, 1940.
10. Lundell, G., and Hoffman, J.: Outlines of Methods of Chemical Analysis, New York, 1938, John Wiley & Sons, Inc., p. 176.
11. Lundell, G., and Knowles, H.: Rapid Detection of Small Amounts of Aluminum in Certain Non-Ferrous Materials, Indust. & Engin. Chem. **18**: 60, 1926.
12. MacKenzie, K.: The Biochemistry of Aluminum. I. Excretion and Absorption of Aluminum in the Pig, Biochem. J. **24**: 1433, 1930.
13. MacKenzie, K.: II. Excretion and Absorption of Aluminum in the Rat, Biochem. J. **25**: 287, 1931.
14. McCollum, E., Rask, O. S., and Becker, J.: A Study of the Possible Role of Aluminum Compounds in the Animal and Plant Physiology, J. Biol. Chem. **77**: 753, 1928.
15. Mull, J. W., Morrison, D., and Myers, V. C.: On the Presence of Aluminum in Blood, Proc. Soc. Exper. Biol. & Med. **24**: 476, 1927.
16. Musakin, A.: Colorimetric Determination of Aluminum With Aluminon, Zavodskaya Lab. **9**: 507, 1940.
17. Myers, V. C., Mull, J., and Morrison, D.: The Estimation of Aluminum in Animal Tissues, J. Biol. Chem. **75**: 595, 1928.
18. Roller, P.: Colorimetric Determination of Aluminum With Aurintricarboxylic Acid, J. Am. Chem. Soc. **55**: 2437, 1933.
19. Schmidt, C., and Hoagland, D.: The Determination of Aluminum in Feces, J. Biol. Chem. **11**: 387, 1912.
20. Underhill, F. P., and Peterman, F.: Studies in the Metabolism of Aluminum. I. Method for Determination of Small Amounts of Aluminum in Biological Material, Am. J. Physiol. **90**: 1, 1929.
21. Führer, J.: Zur Frage der Resorption von Aluminiumverbindungen im Organismus, mit Berücksichtigung des normalen, Aluminiumgehalts tierischer Gewebe, Biochem. Ztschr. **265**: 169, 1933.
22. Yoe, J., and Hill, W.: An Investigation of the Reaction of Aluminum With the Ammonium Salt of Aurintricarboxylic Acid Under Different Experimental Conditions and Its Application to the Colorimetric Determination of Aluminum in Water, J. Am. Chem. Soc. **49**: 2395, 1927.
23. Yoe, J.: Some Observations on Reactions Between Certain Metallic Ions and the Ammonium Salt of Aurintricarboxylic Acid, J. Am. Chem. Soc. **54**: 1022, 1932.

MEDICAL ILLUSTRATION

THE BASIC TECHNIQUE OF KODACHROME PHOTOGRAPHY OF FLUORESCENCE PHENOMENA*

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UNTIL recently the photography of fluorescence phenomena was seriously handicapped since it was unable to reproduce accurately a variety of colors. It was possible to record some of these color differences by means of black-and-white film.[1, 3, 4] However, this method is truly colorless and falls flat when compared with the spectacular and delicate shades of color that have been observed and described as a result of fluorescence in near-ultraviolet radiations. The relatively recent development of practical, efficient sources of near-ultraviolet radiations has given a great impetus to fluorescence studies in many fields, and especially in the field of medical examinations and diagnosis.[1, 2, 4, 5] An accurate method of reproducing colors is the result of the development of Kodachrome film. These two developments of suitable lighting and film have gone hand in hand and each serves to amplify the utility of the other.

The simple efficient apparatus and filters necessary to extend the limited range of visual perception by means of fluorescence phenomena has been reported previously.[2] It is the purpose of this paper to describe and discuss equally simple methods and apparatus for accurately and faithfully recording fluorescence phenomena by means of Kodachrome photography.

Fluorescence is the term applied to the phenomenon which certain substances or bodies show when illuminated with ultraviolet light. It usually involves the absorption of the invisible near-ultraviolet radiations and the emission of visible light of longer wave length. For a more complete discussion, the reader is referred to other literature on this subject.[1, 2, 4]

One of the difficulties that had to be overcome in the photography of fluorescence was the fact that the ordinary film or plate is less sensitive to the rays of visible light caused by fluorescence than it is to the reflected rays of the near-ultraviolet or so-called actinic rays used to excite the fluorescence. The problem was to find a filter to place either in front or in back of the lens of the camera to absorb the reflected near-ultraviolet radiations and to transmit fully the relatively feeble rays of visible light caused by fluorescence. The photography of fluorescence must be done in a dark room. No extraneous or stray light can

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†One of us (F. H. J. Figge) is indebted to Dr. C. N. H. Long and the staff of the Physiological Chemistry Department at Yale University School of Medicine, who first demonstrated the feasibility of Kodachrome photographs of porphyrin incrustations in rats.

This work was aided by a grant from the Anna Fuller Fund for Cancer Research. The reproduction of the Kodachrome photographs was made possible by the J. A. Cox Fund.
Received for publication, December 6, 1941.

be tolerated because of the long exposures necessitated by the low intensity of the light emitted by fluorescent objects. The light source must be intense, especially in the near-ultraviolet range (3,000 to 4,000 A.U.). The primary filter should exclude visible light from the fluorescent object. It should also exclude the far-ultraviolet or tanning and bactericidal rays. This is desirable from the point of view of safety for the photographer and others who may be present.

The photography of fluorescence thus requires, in addition to an ordinary camera, a lamp radiating only ultraviolet or invisible radiations, and a secondary filter which transmits visible light. The desirable characteristics of each of these requirements will be discussed in the course of a description of the apparatus and technique used to take the Kodachrome fluorescence photographs shown in Plate I.

The light source for Kodachrome fluorescent photography may be any type of quartz mercury arc or even carbon arc. Both of these are strong in the near-ultraviolet wave lengths. The primary filter must be applied in such a way as to prevent the leakage of visible light. For this reason the mercury arc is more convenient to use. There are numerous lights on the market today that are designed particularly for fluorescence studies. These have filters built into the lamp, either as close fitting lenses or as an envelope which almost completely surrounds the quartz mercury arc. It is, therefore, advisable to discuss the light source along with the primary filter.

These lamps, with the attached filter, which are designed for fluorescence studies are decidedly superior to many others for photographic utility.* Two of these lamps were used to irradiate the object with near-ultraviolet light in order to obtain the fluorescence photograph in Plate I. One was fixed at a distance of 20 inches from the object, and the other was held in the hand at the same distance and moved from side to side to avoid deep shadows. The bulb of the lamp used consisted of a quartz mercury arc surrounded by a primary filter or envelope of filter glass. This was screwed into a socket on an alzack aluminum reflector. One of the excellent features of this lamp is that it may be moved from side to side or fastened to a ring stand to illuminate the object from any desired angle. It is absolutely necessary to irradiate all sides of an object to be photographed. Due to the mobility of this lamp only one is required, but in order to decrease the exposure time two lamps are advantageous.

The only undesirable feature is that the arc light is enclosed with filter glass similar to No. 587 Corning filter, and this filter is not interchangeable. It transmits a small amount of violet, green, and a trace of red light. Even this almost negligible amount of visible light interferes with the recording of purely fluorescent light. When the light from this lamp was examined with a diffraction grating spectroscope, the violet band at 4,100 A.U. was easily discernible. The red was dim and the green line of the mercury spectrum was only observed when the arc was in line with the slit on the spectroscope. In this connection the Corning filter No. 586 might be more desirable as a primary filter. This is more nearly monochromatic since it transmits no visible light. The near-ultraviolet transmission is, however, much lower than in glasses which transmit traces of visible light. Other Corning filters which are usually recom-

*Detailed specifications will be furnished on request.

mended for fluorescence studies are the red-purple ultra No. 597, red ultra No. 584, blue-purple ultra No. 585, and red-purple corex A No. 986. While these filters are suitable for some studies in fluorescence, they are not suitable for color photography of fluorescence because of the visible light which they transmit.

The secondary filter used to photograph the fluorescence (Plate I) was a Corning glass filter No. 306 Noviol shade O. This has been referred to as the lens filter because it is placed either in front or in back of the lens. The perfect camera lens filter or secondary filter for fluorescence photography has not been found. The ideal filter for this would be one that absorbed all ultraviolet radiations and transmitted all the visible light. The Corning filter No. 038 Noviol shade A was almost ideal, but was itself highly fluorescent. However, this filter has been used successfully by placing it behind the lens inside the bellows where it is protected from most of the near-ultraviolet rays. The Corning filter No. 306 Noviol shade O, although almost ideal, transmits a negligible amount of near-ultraviolet and absorbs a trace of violet and blue. The Wratten No. 2A gelatin filter, which is used most frequently and is recommended by Walter Clark[3] for fluorescence photography, is also good. This does transmit some near-ultraviolet, but absorbs less violet and blue in the visible spectrum than the Corning No. 306. The Wratten No. 2 Aesculine gelatin filter also absorbs ultraviolet radiations shorter than 3,800 to 3,900 A.U. but this, like the Corning No. 038 Noviol A, is intensely fluorescent. When the primary filter is a glass with characteristics of the Corning No. 587 red-purple ultra, the Corning No. 306 is superior to all other secondary filters since it absorbs a part of the purple light transmitted by the primary filter.

The photograph illustrated was taken with a 5 by 7 inch extension bellows camera equipped with a lens having a focal length of 6½ inches. Kodachrome film, 3¼ by 4¼ inches, was used in a reducing back. Similar pictures have also been taken with several other cameras, including a Leica. In general, it may be said that almost any ordinary camera may be used for fluorescence photography. A quartz lens is not required and, as a matter of fact, would be a disadvantage because this would readily transmit the ultraviolet radiations. The glass lens in an ordinary camera also transmits some of the longer wave lengths or near-ultraviolet, but less than the quartz lens would transmit. The camera lens filter is thus necessary to absorb the reflected near-ultraviolet radiations (so-called actinic rays) which are not absorbed by the glass lens.

There is little information available with regard to the fluorescence of camera lenses. It is known, however, that the fluorescence characteristics of glass change with age, and that optical glasses or lenses from different manufacturers show unmistakable variations in fluorescence.[4] The differences in the color of the fluorescent light emitted by various lenses range from pale yellow to violet, blue, and brilliant white. The differences in shade have been described as subtle but so characteristic that glasses from numerous sources almost never fluoresce similarly, and this fact has been used by one manufacturer to distinguish between his own and other products.[4]

The discussion of the fluorescence of glass has been introduced to emphasize the importance of checking this feature in every camera lens before attempting to use it for fluorescence photography. During the long exposure necessary for

J. LAB. & CLIN. MED.
VOL. 27, NO. 12, SEPTEMBER, 1942

A. B.

Fig. 1.

Fig. 2.

A. B.

PLATE I

Fig. 1 *A* and *B*.—Illumination. Visible light. *A*, Normal rat with the harderian gland exposed by removal and displacement of the eyeball. *B*, Rat maintained on a diet deficient in pantothenic acid. Note the incrustation of material that resembles dried blood. Illumination, incandescent tungsten filament (500 watt) at 5 feet. Camera, 5 by 7 inch extension bellows with a lens having a focal distance of 6½ inches. Kodachrome type B film. Exposure time, 2½ minutes at f:45.

Fig. 2 *A* and *B*.—Same as Fig. 1 *A* and *B*, but with near-ultraviolet illumination. Note the red fluorescence of the harderian gland and the porphyrin incrustations. Illumination, two 100 watt G. E. BH4 mercury arc lamps with an envelope of Corning No. 587 filter glass at 20 inches from the rats. Camera, same as above, 18 inches from rats to camera lens. Kodachrome daylight type film. Secondary filter, Wratten No. 2A for entire exposure. Corning No. 430 dark blue-green filter for last 4 minutes of exposure. Exposure time, 12 minutes at f:8.

Kodachrome photography a camera lens that fluoresced appreciably would cause fogging of the film or plate. This point was checked before making the color photograph in Plate I. The lens was not fluorescent so the filter to absorb the near-ultraviolet was placed between the lens and the plate. Had the lens been found to fluoresce, the filter would have been mounted in front of the lens. This would, therefore, have served a double purpose. It would have prevented ultraviolet radiation from reaching either the lens of the camera or the photographic plate. To test a lens for fluorescence irradiate it with near-ultraviolet light. If it is distinctly fluorescent, the lens will be seen to glow. Slight fluorescence may be detected by intermittently shielding the lens from the ultraviolet radiations by an ultraviolet-absorbing filter. If there is a glow which disappears when the lens is shielded, the lens is fluorescent. Shielding the lens with a hood or filter decreases the intensity of the fluorescence and the possibility of objectional effects.

There is great variation in the intensity as well as the wave length of the fluorescent light emitted by different materials. These widely varying conditions make it impossible to recommend specific types of Kodachrome film for recording fluorescence. In general, it may be stated that Kodachrome professional daylight type film is superior for objects which fluoresce a blue white, or blue and green with a little red. For objects that fluoresce more intensely in the red or yellow end of the visible spectrum, the type B professional Kodachrome gives the best color balance. Likewise, no specific rule can be given for the exposure time, except that it must always be relatively long in comparison to exposures with reflected light, because the intensity of the visible light resulting from the fluorescence is low and varies in different objects. Several factors will influence this variable fluorescence intensity in a definite way. The intensity of the light and its distance from the object regulate the intensity of fluorescence. The distance of the object from the camera or film must also be taken into account in estimating the exposure time.

As a prelimanry procedure to determine the proper exposure, a series of test exposures of five, ten, twenty, and forty minutes at f:8 may be made and from these results the proper exposure is approximately estimated. It is imperative in Kodachrome photography to have the proper exposure, since this determines the accuracy of the method of recording the colors.

The fluorescence of the background is also an important feature. The background should be either nonfluorescent or should fluoresce a color that will set off or emphasize the color of the fluorescent object. A dull blue or black (nonfluorescent) background is best for red fluorescent objects. A white or red fluorescent background almost completely masks the fluorescence of red fluorescent objects.

The wide variation in the intensity of fluorescent light from different materials makes it impractical to try to photograph more than one specimen at a time. If this becomes necessary, groups of specimens should be chosen that exhibit a uniform fluorescence with regard to brightness. If this is impossible, it would be better to try to photograph the objects individually and to combine the photographs. In the case of the photograph of the fluorescence of rat fur

and the harderian gland, both objects fluoresce brightly. The red fluorescence of the harderian gland is relatively more intense than the blue-green fluorescence of the fur. It was found that when the proper exposure (twelve minutes) was given for the fur, the intense red of the gland and porphyrin incrustations was overexposed. To avoid this the exposure was made in the usual way for eight minutes. At the end of that time a Corning No. 430 dark shade blue-green filter was introduced between the object and the lens for an additional four minutes. This filter transmits the blue-green light from the fur but practically none of the red from the gland and porphyrin. In other words, the blue-green light from the fur acted on the film for twelve minutes, while the red of the gland and porphyrin incrustations acted only eight minutes. This yielded a more nearly perfect exposure for both materials. Accordingly, it may be seen that it is possible to compensate for differences in the brightness of the fluorescence of two objects by means of colored filters applied during a certain fraction of the exposure. It should be emphasized, however, that this is possible only for two or three different materials or colors, and in cases where the fluorescence spectra do not overlap too much. A number of attempts to compensate for the variations in brightness by means of illuminating the objects with filtered visible light of low intensity proved unsuccessful.

SUMMARY

A Kodachrome photographic method is outlined for recording the more delicate shades of colors caused by the fluorescence of objects when subjected to near-ultraviolet radiation. Some form of quartz mercury arc is recommended as the light source. This light must have a primary filter (Corning glass filter No. 586 or No. 587) transmitting the near-ultraviolet light, and absorbing all or almost all of the visible light. Only two of the secondary or lens filters mentioned in this discussion are suitable and practical. They are the Corning No. 306 Noviol O and the Wratten No. 2A. These may be mounted either in front of or in back of the camera lens. When it is necessary to photograph objects exhibiting the fluorescence of different colors and intensities, a more nearly perfect exposure is attained by means of a colored filter which absorbs the color emitted by the object which fluoresces most intensely. Such a filter would transmit the colors emitted by the materials which fluoresce less intensely. This filter is applied only during the last third of the exposure. Some of the requirements of the camera and lenses are also discussed.

Through the use of these methods and principles, it is possible to record faithfully on Kodachrome film some of the striking color effects brought to light by fluorescence studies.

REFERENCES

1. Danckwortt, P. W.: Lumineszenz-Analyse im Filtrierten Ultravioletten Licht, ed. 3, Leipzig, 1934, Akademische Verlagsgesellschaft m. b. H.
2. Figge, F. H. J.: Near-Ultraviolet Rays and Fluorescence Phenomena as Aids to Discovery and Diagnosis in Medicine, Bull. School Med., Univ. Maryland **26**: 165, 1942.
3. Henney, K., and Dudley, B.: Handbook of Photography, New York, 1939, McGraw-Hill Book Company.
4. Radley, J. A., and Grant, J.: Fluorescence Analysis in Ultraviolet Light, ed. 2, New York, 1935, D. Van Nostrand Co.
5. Rorimer, James J.: Marble Sculptures and the Ultraviolet Ray, Bull. Met. Museum of Art **24**: 185, 1929.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RESPIRATORY INFECTIONS, A Medium for Blood Cultures in, Humbert, C. R. Am. Rev. Tuberc. 45: 456, 1942.

The following formula has been found very serviceable:

Bouillon	
Peptone	10 Gm.
Sodium chloride	5 Gm.
Lung infusion	1,000 c.c.
Solid Medium	
Agar-agar	15 Gm.
Sodium chloride	5 Gm.
Lung infusion	1,000 c.c.

If the powdered lung is used, 120 Gm. in 1,000 c.c. of water are soaked overnight in the ice chest. It is then boiled and filtered. This filtrate is used as described above. The reaction is set at 7.8 pH.

This medium has been useful in obtaining blood cultures in respiratory infections where others have failed. It is also used for many other organisms by adjusting the reaction suitable for their growth.

TUBERCULOSIS, Vitamin K in, With Special Reference to Pulmonary Hemorrhage, Levy, S. Am. Rev. Tuberc. 45: 377, 1942.

Vitamin K and its relation to pulmonary tuberculosis and pulmonary hemorrhage are discussed.

Bleeding time, coagulation time, clotting power, and liver function tests were carried out in four groups of patients with pulmonary tuberculosis and in one control group.

Of the patients with pulmonary tuberculosis 32 per cent had a more or less marked degree of hypoprothrombinemia, while there was no evidence of this condition among the subjects of the control group. Hypoprothrombinemia is a frequent finding in pulmonary tuberculosis and reaches the lowest values among the group of patients with pulmonary hemorrhage.

Parenchymal liver damage was found to be present in 87 per cent of patients with pulmonary tuberculosis, while 33 per cent of the control subjects showed a slight degree of liver dysfunction.

The effect upon the clotting power of drugs routinely used in pulmonary hemorrhage was studied. It was found that sodium pentobarbital causes a lowering of the clotting power. Morphine, koagamen, and calcium also have a slight beneficial effect on the clotting power, although inferior to that of vitamin K.

It is suggested that vitamin K be used routinely as a prophylactic measure in patients whose x-ray films show cavity formation and who are shown to have a hypoprothrombinemia.

Vitamin K, together with the administration of morphine, appears to be the therapy of choice in the prevention and treatment of pulmonary hemorrhage due to tuberculosis.

BLOOD VELOCITY, An Objective Method of Determining (Fluorescein Method), Fishback, D. B., Gullman, S. A., and Abramson, E. B. Am. J. M. Sc. 203: 535, 1942.

A new objective circulation time is described, using fluorescein.

The data obtained indicate that the method is reasonably simple, not harmful to the patient, and reliable as indicated by comparison with other methods.

This method is of particular advantage in determining the blood velocity in small children, and in comatose, anesthetized, mentally ill, and moribund patients.

In patients with normal hearts or with fully compensated cardiac disease, the circulation time varied from 7 to 15.6 seconds. In another group of patients with cardiac disease undergoing decompensation, the circulation time varied from 16 to 25 seconds, one being 45 seconds.

The test for circulation time is done as follows: Using sterile precautions, an 18 gauge needle attached to a syringe containing 4 c.c. of a 10 per cent, or preferably 3 c.c. of a 20 per cent, sodium fluorescein solution is inserted into the antecubital vein of the arm. The room is then darkened, and the fluorescein is injected rapidly into the vein. The assistant, using a portable ultraviolet source with a Wood's filter, directs the light to one of the eyes of the patient, observing the palpebral conjunctiva. The first appearance of a brilliant yellow color in the lower palpebral conjunctiva is the end point. The end point is sharp, especially if the room is dark. The time elapsed from the moment of injection to the appearance of the dye in the eye is recorded.

Thus, the circulation time from the antecubital vein of the arm to the conjunctiva of the eye is accurately determined.

SYPHILIS, Value of Complement Fixation and Agglutination With Spirochetal Antigens and Relation of Spirochetal Antibody to the Wassermann Reaction, Kolmer, J. A. Arch. Dermat. & Syph. **45**: 455, 1942.

Complement fixation tests conducted with antigens of cultivated *S. pallida*, especially of the Reiter and Kazan strains, compare favorably in sensitivity with the Wassermann and flocculation tests in the serologic diagnosis of syphilis of human beings and rabbits, but their practical value in the diagnosis of the disease in its different stages and in relation to treatment has not been sufficiently defined.

Spirochetal complement fixation tests in syphilis of human beings and rabbits are not as specific as the Wassermann and flocculation tests, largely because of the presence in normal serum of natural spirochetal antibody.

This natural antibody, as well as that produced in syphilis, is of a group character reacting not only with cultivated *S. pallida* but with *S. macrodentium*, *S. microdentium*, and other spirochetes.

Spirochetal complement fixing antibody is increased in malaria and in leprosy, with a high incidence of positive complement fixation reactions in both diseases, especially in the former. Under the conditions it is improbable that the spirochetal complement fixation test will identify as biologic false reactions the positive Wassermann and flocculation reactions observed in nonsyphilitic persons with leprosy and malaria.

Normal human and rabbit sera also contain a natural group agglutinin for cultivated *S. pallida* and other spirochetes. The agglutinin undergoes some increase in syphilis but not sufficient for rendering the spirochetal agglutination test with cultivated spirochetes of diagnostic value in syphilis.

The results of agglutination and complement fixation tests with suspensions of virulent *S. pallida* obtained from acute testicular syphilomas of rabbits have indicated more definitely the production of these antibodies in syphilis of human beings and rabbits than tests employing suspensions of the cultivated spirochetes, which suggests that the latter have undergone dissociation into variants with a change in antigenic structure associated with a loss of virulence.

Complement fixation and agglutination tests with cultivated spirochetes have indicated the possible existence of serologic strains of *S. pallida* and also their biologic relation to *S. macrodentium*, *S. microdentium*, and *S. refringens*.

Largely on the basis of absorption of syphilitic human serum with tissue lipids and suspensions of cultivated *S. pallida*, the preponderance of evidence is in favor of regarding the Wassermann reagin and spirochetal antibody as separate entities which may coexist in serum.

Undoubtedly the lipids of *S. pallida* are important in complement fixation by the Wassermann reagin. Whether spirochetal antibody reacts with them is unknown, but it

has been suggested that it fixes complement with some other constituent of spirochetes of a nonprotein nature.

The practical value of antigens of cultivated *S. pallida* in the serologic diagnosis of syphilis and in relation to the treatment of the disease cannot be stated at present, but they are worthy of further study. If such strains are employed, antigens of whole spirochetes in phenolized saline solution appear to be advisable. The Reiter or Kazan strains are preferred, and a mixture of the two may be advisable. The amount employed should be the minimum, giving positive reactions with human syphilitic serum, in order to avoid falsely positive reactions with natural spirochetal antibody as much as possible. Suspensions of virulent tissue spirochetes are preferred, but owing to technical difficulties in their preparation they probably cannot be employed.

CULTURE, Differential, Simple Method of Using Penicillin, Tellurite and Gentian Violet for, Fleming, A. Brit. M. J., May 2, p. 547, 1942.

By the simple method of spreading a small quantity of gentian violet over half of the culture plate of blood agar after it has been planted, it is possible to isolate a few streptococci from the midst of a multitude of staphylococci, just as it has been shown to be if the gentian violet is incorporated in the medium.

Whatever chemical is used with this technique, it is necessary, when spreading it over half the plate, to start where the inoculum is lightest and proceed toward where it is heaviest; otherwise there may be so many resistant bacteria that a confluent sheet of growth results.

Three chemicals can be used in this way with great advantage in the clinical bacteriological laboratory. They are penicillin, potassium tellurite, and gentian violet.

The great advantages of this simple method of spreading the chemical over half the culture plate after inoculation are (1) special media need not be prepared; (2) one-half of the culture plate is an ordinary culture and the other half a selective culture, thus really giving the effect of two culture plates in one.

GLUCOSE, The Assimilation Rate of Intravenously Injected, in Hospital Patients, Cain, J. C., and Belk, W. P. Am. J. M. Sc. 203: 359, 1942.

The assimilation rate of intravenously administered glucose in hospital patients was found to be correlated with the rate of injection. The assimilation of 5.77 Gm. per kilogram of body weight per hour was observed.

In all cases some glucose was lost in the urine, the amounts varying from approximately 0.3 to 30 per cent of the total given. The average loss was 12.2 per cent.

The proportion of injected glucose lost in the urine was not correlated with the rate of injection, nor with the total amount given within the limits studied. This "spill" is conditioned by factors within the individual patients.

For practical purposes the rate for the intravenous administration of glucose may be determined solely on two considerations; first, the hydrodynamics of the patient's circulation; second, technical convenience.

SYRINGE, Removing "Frozen" Plungers by a Hydraulic Pressure Method, Novak, M., and Lacy, A. M. Am. J. Technol. 8: 42, 1942.

A 1 inch No. 22 Yale hypodermic needle is telescoped into a No. 19 (1½ inch) Yale needle so as to make a firm connection. One needle is attached to the frozen syringe, and the other to the tuberculin syringe which is filled with water. By forcing water into the "frozen" syringe, pressure is exerted on the "frozen" plunger and almost always it will be forced out. Air in the system should first be displaced with water for best results.

This simple device may be made in a minute from old needles, and kept on hand for use when needed. Any combination of needles may be used as long as they fit together firmly. The use of the two needles in this manner makes a flexible connection and diminishes the chances of breaking the ends of the syringes.

TISSUE, Combined Frozen and Paraffin Method for Rapid Sections, Thomlinson, B. C. Arch. Path. 33: 35, 1942.

Technique

1. The tissue, if not already fixed, is placed in hot dilute formaldehyde solution (10 c.c. of solution of formaldehyde U.S.P. in 90 c.c. of water) for three minutes. The solution should be steaming (about 65° C.) but not boiling, and the pieces of tissue may be large, but not over 3 mm. in thickness.

2. Frozen sections, as thin as possible (10 to 15 microns), are prepared by the usual method. Overfreezing should be avoided since it causes an unnecessary delay.

3. Sections are transferred from the knife to a dish of warm (40° C.) 0.2 per cent gelatin solution, from which a section is then floated carefully onto a clean slide, and any wrinkles which may form are teased out.

4. The excess fluid around the section is wiped off, and the slide is dried cautiously over the pilot flame of a Bunsen burner. Overheating is to be avoided.

5. When the section is thoroughly dried, it is cooled and placed in formaldehyde alcohol (10 c.c. of solution of formaldehyde U.S.P. in 90 c.c. of 95 per cent ethyl alcohol) for one-half minute.

6. It is then dehydrated in 95 per cent ethyl alcohol, followed by absolute alcohol, one-half minute in each.

7. After being cleared in xylene for one-half minute, it is transferred to a beaker of melted paraffin in an oven at 60° C. for three minutes.

8. The slide is then removed from the beaker of paraffin and returned to the oven for five minutes. This assures attachment of the section to the slide during the staining process and apparently produces a mordant effect on the tissues.

Various staining methods may now be employed. The technique for the hematoxylin-eosin method is as follows:

1. Remove paraffin with xylene for 30 seconds.
2. Place in 95 per cent alcohol for 15 seconds.
3. Place in 70 per cent alcohol for 15 seconds.
4. Wash quickly in tap water for 10 seconds.
5. Stain in fresh Harris hematoxylin for one minute.
6. Wash in water, and decolorize in 0.5 per cent aqueous solution of hydrochloric acid. Wash and intensify in a weak solution of ammonia water and again wash for 30 seconds.
7. Counterstain with eosin and rinse in water for one minute.
8. Dehydrate in 70 per cent, 95 per cent, and absolute alcohol for 10 seconds each.
9. Clear in xylene.
10. Mount in Canada balsam.

HEMOSIDERIN, A New Modification of Perls's Reaction for, in Tissues, Highman, B. Arch. Path. 33: 937, 1942.

It is suggested that one volume of a 2 per cent aqueous solution of potassium ferrocyanide be mixed with an equal volume of a 4 to 6 per cent aqueous acetic acid, heated in a test tube until beads of gas appear on the inner surface of the glass (about 60° to 80° C.), poured on the section, and allowed to remain thereon for from forty-five seconds to five minutes.

After being washed in water, the section is counterstained for five minutes with a 1:6,000 to 1:10,000 solution of basic fuchsin in 2 per cent aqueous acetic acid.

Index photographed at the
beginning for the convenience
of the microfilm user.